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Shifts in the estuarine demersal fish community after a fishery closure in Puget Sound, Washington

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Abstract—Puget Sound is one of the largest and most ecologically significant estuaries in the United States, but the status and trends of many of its biological components are not well known. We analyzed a 21-year time series of data from standardized bottom trawl sampling at a single study area to provide the first assessment of population trends of Puget Sound groundfishes after the closure of bottom trawl fisheries. The expected increase in abundance was observed for only 3 of 14 species after this closure, and catch rates of most (10) of the abundant species declined through time. Many of these changes were stepwise (abrupt) rather than gradual, and many stocks exhibited changes in catch rate during the 3-year period from 1997 through 2000. No detectable change was recorded for either temperature or surface salinity over the entire sampling period. The abrupt density reductions that were observed likely do not reflect changes in demographic rates but may instead represent distributional shifts within Puget Sound.

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Estuaries support diverse marine communities and act as nursery areas for many coastal populations (Beck et al., 2001; Armstrong et al., 2003), but they are the most heavily affected marine ecosystems (Lotze et al., 2006; Halpern et al., 2008) because they also commonly serve as ports for shipping, support commercial and recreational fisheries, and are used as recreational areas. Because of their close link with terrestrial systems, they are susceptible to coastal eutrophication (Carpenter et al., 1998) that leads to hypoxia (Diaz, 2001; Breitburg et al., 2009), harmful algal blooms (Anderson et al., 2002), and concentrations of contaminants (Nichols et al., 1986). Because of the myriad ecosystem services they provide (Guerry et al., 2012) and the many human activities that may impair their delivery, there is a growing effort to protect and restore these ecosystems. However, assessment of the efficacy of protection measures is often hindered by the lack of long-term, standardized data and by confounding changes in many aspects of the ecosystem, such as fishery management, shoreline protection, and water quality.

Puget Sound is one of the largest and most ecologically significant estuaries in the United States, supporting a rich fauna with more than 200 fish species, 26 marine mammals, more than 100 bird species and a high diversity of invertebrates.[1] It is the second-largest estuary (2330 km^2) in the coterminous United States, and its watershed supports a large and growing human population. Land alteration and habitat loss (Levings and Thom, 1994), fishing,[2] and toxic contaminants (Landahl et al., 1997) have had widespread effects on this ecosystem. Currently, 8 fish species or fish stock in Puget Sound are protected under the U.S. Endangered Species Act, and many others are identified as being at risk (Musick et al., 2000). Tagging studies, genetic analyses, and differences in toxic contaminant levels all indicate that Puget Sound stocks of various fish species are distinct from coastal stocks (Day, 1976; Andrews et al., 2007; West et al., 2008; Andrews and Quinn, 2012). Notwithstanding these issues, much of the area

[1] Ruckelshaus, M. H., and M. M. McClure. 2007. Sound science: synthesizing ecological and socioeconomic information about the Puget Sound ecosystem. U.S. Dep. Commer., NOAA, National Marine Fisheries Service, Northwest Fisheries Science Center. Seattle, WA. 93 p. [Available from http://www.nwfsc.noaa.gov/research/shared/sound_science/documents/sound_science_finalweb.pdf.

[2] Palsson, W. A., T. J. Northrup, and M. W. Barker. 1998. Puget Sound groundfish management plan, 48 p. Washington Department of Fish and Wildlife, Olympia, WA. [Available from http://wdfw.wa.gov/publications/00927/.]

in Puget Sound is deep because of its glacial origin (Burns, 1985), compared with the much shallower estuarine systems on the East Coast of North America. Therefore, the shallow biogenetic habitats that have been altered by humans—a common occurrence in estuaries—encompass a relatively small fraction of the total available habitat. The local government is working to assess the status of the Puget Sound ecosystem and to identify and implement measurable restoration goals.[3] However, this planning process is hindered by a paucity of long-term data on species and community trends.[4] Without such time series, it is difficult to assess the rate and extent of recovery that may be reached within planning timelines.

Here we present our analysis of one of the longest continual and standardized surveys of the groundfish community in Puget Sound (surveys conducted by the University of Washington) to assess the nature and extent of change that has accompanied significant restoration measures. Most significantly, the state of Washington progressively prohibited commercial trawling by closing most waters of central and southern Puget Sound in 1989, and then closing all inland marine waters to nontribal bottom trawling in 2010.[2] Therefore, we hypothesized that these survey data—the collection of which began in 1991—would provide an indication of rates of recovery of exploited fish stocks and community reorganization because not all species were exploited. Our time series is limited in spatial extent but provides a 20-year record of species composition and abundance of the groundfish community and, therefore, may indicate the effect of commercial trawling and enable assessment of the status of recovery.

This ecosystem affords a rare opportunity to track the recovery of groundfish populations and communities in response to a commercial fishery closure. Typically, information about fisheries effects has come from tracking changes in "no-take" marine reserves (Russ and Alcala, 1996; Babcock et al., 1999; Halpern, 2003). Although such spatial closures provide important information for identifying restoration targets, no-take areas are often smaller in area than the range of populations affected by fishing and, therefore, may not reveal the full extent of fishing effects (Claudet et al., 2008). In contrast, the commercial trawl-fishing closure in Puget Sound covered a large area that closely matches the distribution scales of resident populations. Moreover, because more than 2 decades have passed since the closure, we have the potential to describe not only the extent but also the trajectory of population recovery. Therefore, we can ask whether recovery was monotonic as predicted by simple population models or whether, instead, it was characterized by abrupt and sustained shifts in abundance and composition that would indicate either nonlinear population or community dynamics (Doak et al., 2008; McClanahan et al., 2011) or decadal-scale environmental drivers (Mantua et al., 1997; Anderson and Piatt, 1999).

Our specific objectives were to determine 1) whether catch rates of resident Puget Sound groundfishes generally increased through time following the closure of bottom trawl fisheries, 2) the extent to which shifts in this time series may represent population fluctuations or instead represent local effects that result from distribution shifts, 3) whether dynamics are best represented by smooth trends through time or instead though more abrupt state-changes, and 4) whether observed trends in catch rates for resident Puget Sound groundfish populations may be linked to changes in environmental conditions reflected in oceanographic monitoring data.

Materials and methods

Study location and design

Catch data were derived from bottom trawl surveys conducted in Port Madison, a large bay on the west side of central Puget Sound, north of Bainbridge Island (Fig. 1; see also Andrews and Quinn [2012] for specific sampling sites) as part of **a** Fisheries Ecology course of the University of Washington. The study area is located in the central basin of Puget Sound, the largest of the 4 main basins that compose the inland marine waters of Washington State. The area is not industrialized, and the shoreline is primarily a natural bluff-beach formation typical of central Puget Sound with some armoring around private residences. All sampling was conducted on the third weekend in May, beginning in 1991 and continuing until 2012; sampling did not occur in 1992 and 1998.

Bottom trawl surveys consisted of single tows of ~5 min conducted at 4 fixed index sites at discrete depths (10, 25, 50 and 70 m) over 5 diel time periods: afternoon (~15:00–18:00 h), evening (~20:00–23:00 h), night (~01:00–04:00 h), morning (~06:00–09:00), and mid-day (~11:00–14:00 h). This survey design was intended to capture and account for diel shifts in onshore–offshore distribution of key species (Andrews and Quinn, 2012). Trawl paths did not overlap within sampling years but were staggered slightly. All sampling was conducted from RV *Kittiwake*. Each tow covered 0.37 km at 0.5 m/s, with a standard Southern California Coastal Water Research Project bottom trawl that had a footrope

[3] Puget Sound Partnership. 2009. Puget Sound Action Agenda : Protecting and restoring the Puget Sound ecosystem by 2020, 213 p. Pugest Sound Partnership, Olympia, WA. [Available from http://www.psp.wa.gov/downloads/AA2009/Action_Agenda_FINAL_063009.pdf.

[4] Essington, T. E., T. Klinger, T. Conway-Cranos, J. Buchanan, A. James, J. Kerschner, I. Logan, and J. West. 2011. The biophysical condition of Puget Sound. *In* Puget Sound Science Update, p. 205–423. Puget Sound Partnership, Tacoma, WA. [Available from http://www.psp.wa.gov/scienceupdate.php.]

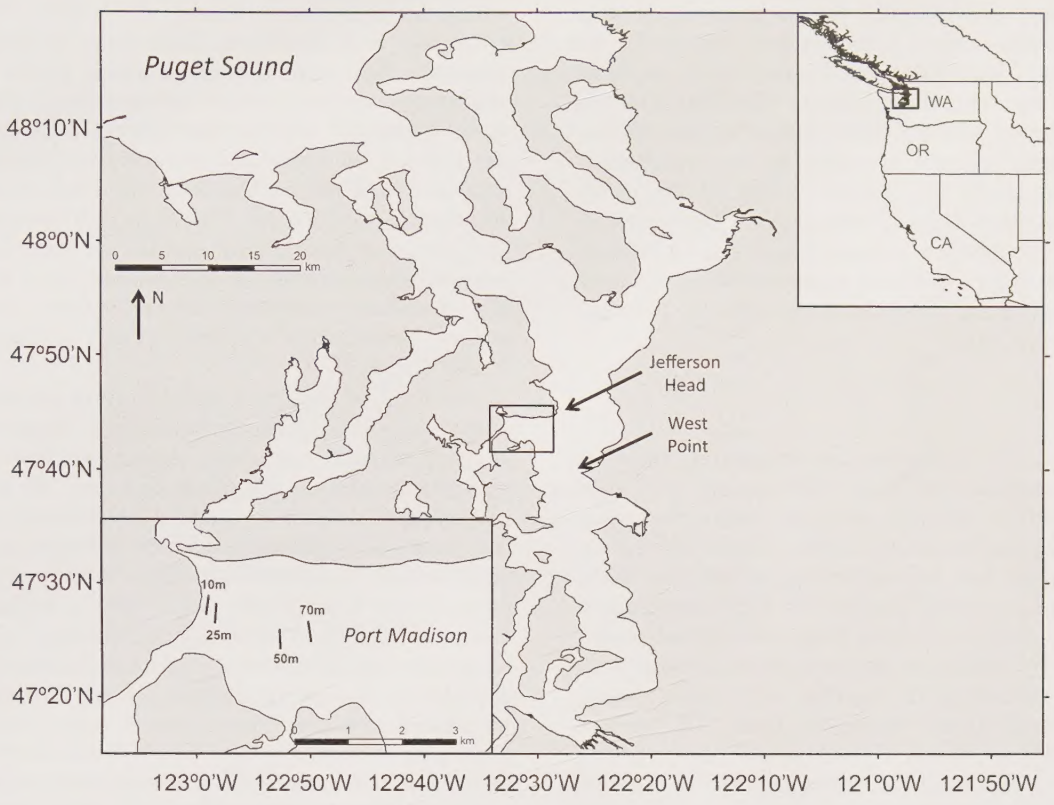

Figure 1

Map of the locations of the study area (rectangle at the center) where bottom trawl surveys were conducted in Port Madison from 1991 to 2012 and of 2 nearby monitored sites (labeled West point and Jefferson Head) where time series data on environmental conditions, such as temperature and surface salinity, were recorded. Map inset in upper-right corner shows location of Puget Sound, Washington, along the U.S. Pacific coast, and map inset in lower-left corner shows detailed view of the Port Madison study area and locations of 4 sampling sites with corresponding depths listed.

of 5 m and net width of 3.5 m during fishing.[5] The bottom trawl was fitted with a 3.8-cm body mesh and 3.2-cm codend mesh with a 0.4-cm codend liner. The net primarily targets flatfishes but also catches small demersal fishes, such as gadids and some elasmobranchs.

Fish were identified to species on deck with the aid of dichotomous keys (Hart, 1973), but a few individuals were retained for examination in the laboratory. We measured fork length for all species except length of Spotted Ratfish (*Hydrolagus colliei*), for which precaudal length (tip of snout to second dorsal fin; Anderson and Quinn, 2012) was measured; all length measurements were made to the nearest millimeter. Consistency in field identification was facilitated by the presence of one of us (T. Quinn) for virtually every tow in the entire time series.

[5] Eaton, C. M., and P. A. Dinnel. 1993. Development of a trawl-based criteria for assessment of demersal fauna (macroinvertebrates and fishes): pilot study in Puget Sound, Washington, 87 p. Final report to the U.S. Environmental Protection Agency. Bio-Marine Enterprises, Seattle ,WA.

Environmental data

We obtained data from 2 monitoring sites on water-column characteristics (temperature and salinity profiles) for March, April, and May. The King County Water and Land Resources Division samples a location 4.4 km northeast of Port Madison called Jefferson Head, and the Washington State Department of Ecology samples a location 8.5 km southeast of Port Madison called West Point—both on a monthly basis (Fig. 1). We used data from both of these sampling programs (1990–2008 for West Point, 1992–2008 for Jefferson Head) to identify years and time periods with unusual environmental conditions on the basis of sub-mixed-layer temperature and surface salinity. Surface salinity gives a measure of seasonal runoff and, therefore, indicates seasonal weather events (years with high precipitation have low surface salinity). Sub-mixed-layer temperature is indicative of the thermal habitat experienced by groundfishes. Sub-mixed-layer temperature was used instead of bottom temperature because the latter was not always sampled. When bot-

tom temperature was sampled, it was generally within 0.7°C of sub-mixed-layer temperature (depth=20 m) for March, April, and May. We focused on data from these months because they include the time periods immediately before bottom trawl sampling and, therefore, could best indicate changes in environmental conditions that might affect catch rates. Moore et al. (2008) demonstrated strong intra-annual coherence of oceanographic properties within Puget Sound basins; therefore these data are likely representative of intra-annual environmental conditions throughout the central Puget Sound basin.

Analysis

For most years, all 20 depth×time combinations were successfully sampled, but gear malfunction and other events resulted in missing sets for some sampling sites. These missing sets constituted only 5% of the total sample design, but we wanted to account for them in deriving annual catch levels. We first ascertained whether these differences can alter annual estimates of catch rates by fitting an analysis of variance (ANOVA) for each of our study species with year, depth, time, and a depth×time interaction term. All but one species, the Shiner Perch (*Cymatogaster aggregata*), showed either a significant effect of depth, depth+time, or a depth×time interaction term.

We used a simple approach to account for the small numbers of missing sets. Rather than fitting generalized linear models to calculate a statistical "year effect," we instead calculated an annual average catch anomaly for each year on the basis of expected catches for each time×depth combination. This approach is equivalent to fitting a generalized linear model with a time+depth+time×depth interaction term, but it has a straightforward interpretation and permitted a parallel calculation for the trawl and environmental data. We calculated the mean catch rate (number of fish/tow) for each depth×time combination for each species with data from the entire sampling period. We then calculated the catch anomaly as the difference between observed species-specific catch and the expected (mean) catch rate given the depth and time of sampling. The annual abundance index for each species was equal to the average catch anomaly over all samples conducted within a year. We used the same approach to generate temperature and salinity anomalies for each year. For each month and monitoring site, we calculated the mean temperature and salinity values from all available data, generated anomalies for each year, month, and site, and averaged these across months to derive a yearly anomaly value.

We generally tracked abundances at the species level, but, in some cases, we aggregated closely related species. Rock soles were allocated to a single species when the survey began, but subsequent genetic work indicated that the rock sole genus (*Lepidopsetta*) consists of 3 species (Orr and Matarese, 2000), 2 of which occur in Puget Sound: Rock Sole (*Lepidopsetta bilineata*) and Northern Rock Sole (*Lepidopsetta polyxystra*). We conducted our analysis at the scale of an aggregated species group because the 2 Puget Sound species are not readily distinguished in the field and we wanted to maintain consistency throughout the time series. Further, Speckled Sanddab (*Citharichthys stigmaeus*) and Pacific Sanddab (*Citharichthys sordidus*) are morphologically similar as juveniles; for this reason, species-level identifications were not reliable. We, therefore, combined all individuals identified as either species into a species group termed "sanddab; (*Citharichthys*)."

We focused analysis on the most common species and species groups encountered with the sampling gear so that we had sufficient statistical power to detect changes in abundance through time. We set an arbitrary threshold of 200 sampled individuals over the entire time period for species to be included in the analysis. This use of a threshold eliminated species so rarely encountered that trends would not be reliable, species for which the gear was not appropriate, and samples for which species identity could not be determined (e.g., samples in very early juvenile stages). For each species, we asked whether abundance changed through time, and, if so, whether it was best described by a continuous linear increase or decrease or a discontinuous shift in the mean catch rate. The latter is consistent with regime shifts as reflected by rapid and persistent changes in population densities (Rodionov and Overland, 2005). For each time series, we used Akaike's information criteria adjusted for a small sample size (AIC_c) to choose between 3 models: constant, linear, or change point. For each model, we assumed normally distributed residuals. We used the changepoint package (vers. 0.6; Killick and Eckley, 2011) in R software (vers. 2.13; R Development Core Team, 2011) to assess discontinuous shifts in the mean catch rate. We required that the best fitting change-point model consist of time periods spanning at least 4 years of data. In other words, estimated change points that broke the time series into increments shorter than 4 years were discarded, thus preventing the model from placing change points at the beginning or end of time series.

Because we found evidence of change points for many flatfishes, we explored the data for flatfish species in more detail. The gear captures individuals across a wide size range and range of life history stages; therefore we evaluated whether changes in catch rate could be attributed to changes in recruitment patterns. If changes in densities were driven by changes in recruitment, we would expect to see time trends of abundance for small size classes to lead trends for larger size classes. For each flatfish species, we calculated catch anomalies separately for small and large size classes (individuals below the 33rd percentile and above the 66th percentile of the cumulative length-frequency distribution, respectively). Size-at-age data are not available for most species, but for English

Table 1

Summary of total catches for species or species groups that were commonly collected during bottom trawl surveys conducted from 1991 to 2012 in Port Madison, Puget Sound, Washington. A full listing of all species collected is presented in the appendix table.

Species	Catch (number of fish)	Percentage of total
Blackbelly Eelpout (*Lycodes pacificus*)	3398	9.3
Dover Sole (*Microstomus pacificus*)	366	1.0
English Sole (*Parophrys vetulus*)	10,427	28.6
Flathead Sole (*Hippoglossoides elassodon*)	1665	4.6
Pacific Hake (*Merluccius productus*)	958	2.6
Pacific Herring (*Clupea pallasii*)	478	1.3
Pacific Tomcod (*Microgadus proximus*)	2677	7.3
Plainfin Midshipman (*Porichthys notatus*)	497	1.4
Rock soles (*Lepidopsetta bilineata* and *L. polyxystra*)	773	2.1
Sanddabs (*Citharichthys sordidus* and *C. stigmaeus*)	1075	3.0
Sand Sole (*Psettichthys melanostictus*)	680	1.9
Shiner Perch (*Cymatogaster aggregata*)	1139	3.1
Slender Sole (*Lyopsetta exilis*)	2242	6.2
Spotted Ratfish (*Hydrolagus colliei*)	4068	11.2

Sole (*Parophrys vetulus*), the most common species, available aging data (senior author, unpubl. data) indicate that this procedure effectively separates age-1 individuals from those individuals aged 3 years and older.

Results

During the 20-year survey 65 fish species were sampled, and the 14 species that were sampled frequently enough (>200 individuals) to evaluate time trends accounted for more than 85% of the total catch (Table 1). Notably, 7 of these species were flatfishes (Pleuronectidae and Paralichthyidae). English Sole and Spotted Ratfish were by far the most common species, collectively, contributing more than 40% of all individuals sampled. Other common species included Blackbelly Eelpout (*Lycodes pacificus*) and Pacific Tomcod (*Microgadus proximus*).

Time series of catch anomalies were nonstationary for most species (Fig. 2). Spotted Ratfish was the only species for which no trend or apparent change in abundance over the sampling period was observed (Table 2; Fig. 2). As for changes in abundance values that were seen, a change point was identified for 9 species and a continuous linear trend was found for only 3 species. In all cases where the change-point model provided the best fit to the data, the relationship indicated a reduction in the mean catch rate in the later portion of the time series. These cases included the one for the most abundant species, English Sole, for which the mean catch anomaly shifted from +27/tow before 1998 to –7/tow afterward. In contrast, increases in the mean catch anomaly were observed for all 3 species for which abundance trends were best described by the linear model. Trends in total catch (unstandardized) summed across all species mirrored the trends of English Sole (Fig. 2).

Many species exhibited changes in catch rates at similar time periods. Of the 9 species whose dynamics were best described by a change-point, 5 species had estimated change points between 1997 and 1999 (catches were not sampled in 1998). Three species had change points between 1999 or 2000 and 2000 or 2001. Therefore, there was evidence of a change in catch rates between 1997 and 2001 reflected by several species.

Analysis of catch-anomaly trends among different size classes of flatfishes did not support the hypothesis that trends were driven by changes in recruitment (Fig. 3). For most species, anomalies for small- and large-size fishes were synchronous with no apparent lag. On the basis of the length-frequency distribution of each species or species group, fishes were categorized as small if they fell in the 33rd percentile or lower and as large if they were assigned to the 67th percentile or higher. For instance, the catch anomalies for English Sole were nearly identical between small (age 1) and large (age 3+) size classes. Catch-anomaly trends for the rock sole species group were more consistent with recruitment shifts because catch anomalies of small rock soles declined steeply after 1997 but catch anomalies for large rock soles had a less sudden and delayed decline. For Dover Sole (*Microstomus pacificus*) and sanddabs, nearly all the variation in catch anomaly was attributed to large-size individuals; catches of small individuals changed little.

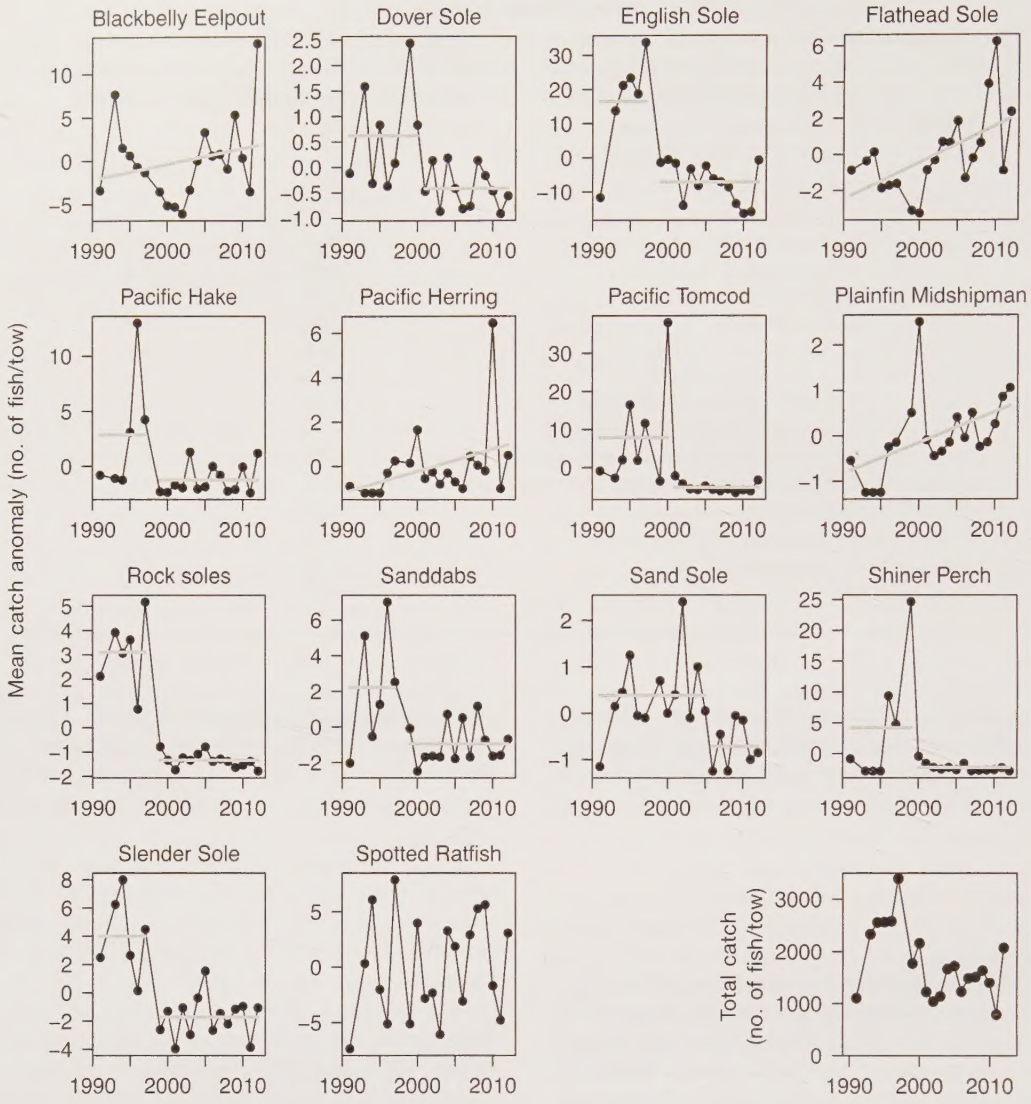

Figure 2

Time series of annual catch anomalies for 14 species or species groups commonly captured during bottom trawl surveys conducted during 1991–2012 in Port Madison, Puget Sound, Washington. Gray lines depict instances where the linear or change-point model provided the best fit to the data. Graph in the bottom right corner depicts total survey catch (unstandardized). Catch anomalies are the yearly averaged differences between each trawl tow and the average catch rate (number of fish) for all tows collected at the same depth and time of day.

Time series of anomalies in sub-mixed-layer temperature and surface salinity were generally consistent with each other between the 2 monitored sites (Fig. 4). The data indicated exceptionally warm years in 1992, 1994, 1998, 2003, and 2004 and cool years in 1993, 1999–2002, and 2008. For each of the 2 sites, temperature time series indicated neither a distinct shift near 1997–1999 nor any other change point or linear trend (Table 3). The time series of surface salinity for the West Point site was generally more variable than the time series for the Jefferson Head site, yet both time series showed the same years as exceptionally high or low. At both sites, 1992, 2001, 2004, and 2008 were high-salinity years and 1991 and 1997 were low-salinity years. There was no indication of a linear trend or change point in the surface salinity at West Point (Table 3), but the surface salinity at Jefferson Head showed a positive linear trend (change of roughly 0.04 ppt/year).

Table 2

Comparison of 3 models of changes in catch rates of species or species groups commonly collected during bottom trawl surveys conducted from 1991 to at Port Madison, Puget Sound, Washington. Akaike's information criteria adjusted for small sample size (AIC_c) was used to choose between the models compared: constant (no change), linear (change through time), or change point (abrupt change at a single point in time, with no temporal change elsewhere). Values indicate a difference in AIC_c for each species from lowest AIC_c among all species. No result is given for species for which the change-point model estimated a breakpoint in the first 4 or last 4 years of the time series and, therefore, these species could not be considered in change-point model comparisons.

	ΔAIC_c		
Species	Constant	Linear	Change point
Blackbelly Eelpout (*Lycodes pacificus*)	1.98	0.00	–
Dover Sole (*Microstomus pacificus*)	31.07	12.95	0.00
English Sole (*Parophrys vetulus*)	65.77	26.91	0.00
Flathead Sole (*Hippoglossoides elassodon*)	28.70	0.00	–
Pacific Hake (*Merluccius productus*)	21.37	16.74	0.00
Pacific Herring (*Clupea pallasii*)	9.62	0.00	–
Pacific Tomcod (*Microgadus proximus*)	30.74	19.15	0.00
Plainfin Midshipman (*Porichthys notatus*)	19.94	0.00	–
Rock soles (*Lepidopsetta bilineata* and *L. polyxystra*)	154.96	82.95	0.00
Sanddabs (*Citharichthys sordidus* and *C. stigmaeus*)	30.06	18.88	0.00
Sand Sole (*Psettichthys melanostictus*)	28.72	23.23	0.00
Shiner Perch (*Cymatogaster aggregata*)	15.22	11.95	0.00
Slender Sole (*Lyopsetta exilis*)	82.67	38.21	0.00
Spotted Ratfish (*Hydrolagus colliei*)	0.00	0.23	–

Discussion

We hypothesized that the data from Port Madison would reveal trends of increasing abundance in resident groundfish populations in Puget Sound after the cessation of commercial bottom trawling and, thereby, would indicate rates and magnitudes of recovery. Before the ban on commercial trawling in the central basin of Puget Sound, commercial catches ranged from 224 metric tons (t)/year to more than 500 t/year and, therefore, likely represented a significant source of mortality for many targeted species.[6] Commercial fish catches through other methods (set nets, purse seines, or set lines) also have been reduced sharply.[5] However, most species exhibited nonlinear patterns of abundance characterized by abrupt and sustained changes in relative abundance indices during the 21-year time period that the survey spanned. These abrupt abundance shifts were notable because they were most commonly in the opposite direction from our expectation and appeared to be synchronous among different common groundfish species. Moreover, these shifts did not appear to be related to demographic changes indicative of recruitment shifts, and they were not linked to temporal patterns in local water temperature and salinity. Currently, no recruitment time series are available for demersal fishes in Puget Sound.

There are several possible explanations for the synchronous reduction in catch rates of groundfish species that occurred in the late 1990s to early 2000s. The first is loss or impairment of habitat that resulted in emigration out of the survey area. Most of these groundfish species reside on soft-bottom habitats (sand or mud) and do not rely on biogenic habitats, such as eelgrass beds, that are particularly vulnerable. However, Nichols (2003) reported an increase in abundance of common prey items of English Sole in Port Madison and in nearby areas from the early 1960s to the early 1990s. It is possible that this trend reversed after this time period, although direct data are needed to evaluate this hypothesis. Alternatively, trawling itself may have altered physical habitat and benthic infaunal communities (Auster et al., 1996); cessation of this activity may have promoted a community of less-preferred prey for these fish predators. Little information, however, is available on bottom habitat or infaunal community dynamics to test any of these hypotheses.

Alternatively, the second explanation is that changes in catch rates in Port Madison may reflect expansions and contraction of population ranges, possibly as a consequence of changes in population densities (MacCall, 1990). However, the sharp decreases in abundance that we witnessed suggest a decline in densities throughout Puget Sound and a contraction to other habitats. This implication is not supported by data from bottom trawl

[6] Schmitt, C. S., S. Quinnell, M. Rickey, and M. Stanley. 1991. Groundfish statistics from commercial fisheries in Puget Sound, 1970–1988. Progress Report No. 285, 315 p. Washington Department of Fisheries, Olympia, WA.

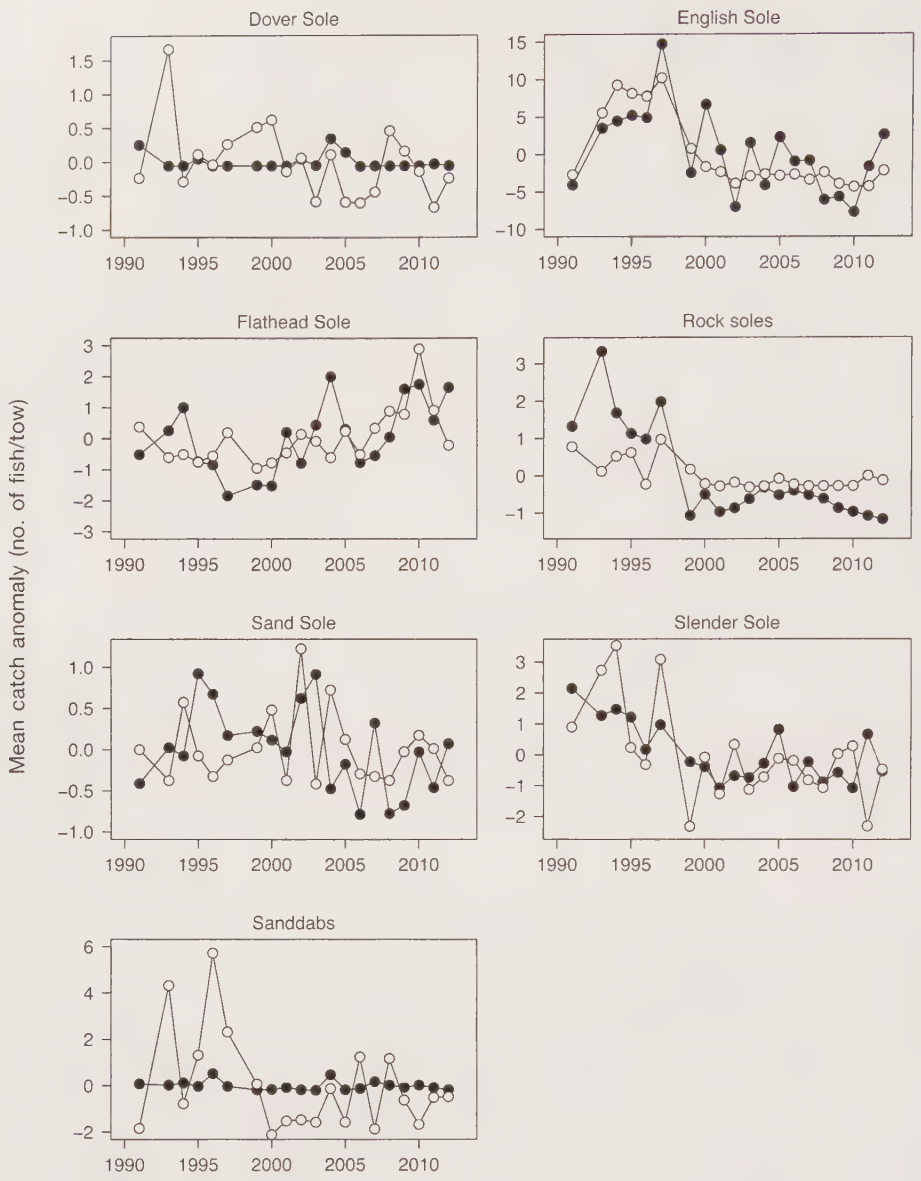

Figure 3

Time series of annual catch anomalies for small (solid circles) and large (open circles) size classes for 7 species or species groups of flatfishes collected during bottom trawl surveys conducted during 1991–2012 in Port Madison, Puget Sound, Washington. Small and large-size individuals were categorized as those fishes in the 33rd percentile or lower and those fishes in the 67th percentile or higher, respectively, on the basis of the length-frequency distribution of each species or species group.

surveys that cover a wider area and that generally indicate greater groundfish densities through the 1990s.[7]

Third, decreases in catch rates could be explained by changes in predator abundance in this region that may impose increased mortality or result in distributional shifts of prey species (Heithaus et al., 2008). Demersal fish species in Puget Sound are consumed by elasmobranchs, such as Spiny Dogfish (*Squalus acanthias*) and Bluntnose Sixgill Shark (*Hexanchus griseus*), and marine mammals, such as the harbor seal (*Phoca vitulina*; Bromaghin et al., 2013; Howard et al., 2013) and

[7] Palsson, W. 2010. Personal commun. NOAA Fisheries, Alaska Fisheries Science Center, Seattle, WA. 98115.

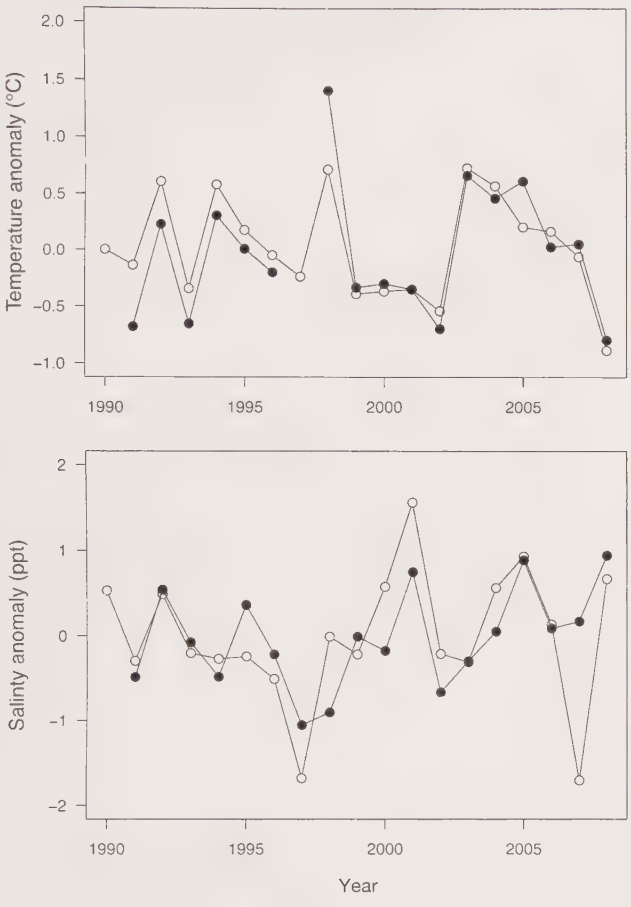

Figure 4

Time series of annual temperature and surface-salinity anomalies for the period of 1990–2008 for 2 monitored sites in Puget Sound, Washington: West Point (gray circles) and Jefferson Head (black circles). Temperature and salinity anomalies were calculated as average deviations from the mean levels for March–May. Temperatures were taken at a depth of 20 m.

California sea lion (*Zalophus californianus*), but we are unaware of any abrupt changes in predator densities in Port Madison to explain these patterns. Jeffries et al. (2003) reported a monotonic increase in harbor seal abundance in Puget Sound from the late 1970s to the 1980s that was followed by little change in abundance during the mid- to late 1990s.

Fourth, groundfish densities can be sensitive to water quality, especially to oxygen concentrations at the seafloor that result in distributional shifts to normoxic conditions (Breitburg et al., 2009; Essington and Paulsen, 2010), and chronic hypoxia exposure could diminish productivity of groundfish prey (Diaz and Rosenberg, 1995). There is no consistent sampling for dissolved oxygen in Port Madison to evaluate this hypothesis, but the exposure of this area to strong tidal currents and subsequent high mixing likely mean that this area is not particularly prone to low dissolved oxygen (Nichols, 2003). Moore et al. (2008) did not detect a change in water temperatures throughout Puget Sound from 1993 to 2002; therefore, changes are unlikely a result of a shift in temperature on a scale larger than Puget Sound.

Several important limitations of our data warrant specific discussion. First, our bottom trawl sampling—although highly standardized in time, space, and method—may not be representative of the entire Puget Sound. Indeed, one of our main conclusions is that shifts in densities of demersal fish species more likely were indicative of distributional shifts than of population shifts. Also, the opening of the bottom trawl was small and, therefore, likely had low selectivity for large-size groundfishes (e.g., >50 cm). Additionally, because sampling was restricted to a standardized and limited time of year, the data cannot account for seasonal changes (Reum and Essington, 2011) and may not reflect trends apparent in different seasons.

Our environmental data were collected from monitoring sites near the study area for bottom trawl surveys, and bottom temperature was not always recorded. We used sub-mixed-layer temperature as a proxy for bottom temperature, which appeared to be robust for years in which bottom data were available. Bottom water temperature may deviate from temperature of the shallower sub-mixed layer because of water exchange between Admiralty Inlet, the Strait of Juan de Fuca, and the coastal Pacific Ocean. However, because deepwater dynamics reach equilibrium over time scales of months, they reflect local, seasonal environmental conditions (e.g., air temperature, freshwater runoff) (Ebbesmeyer and Barnes, 1980) and, therefore, are useful for interannual comparisons. Despite these limitations, this study presents the first long-term standardized assessment of the groundfish community in Puget Sound and, therefore, can provide a baseline for expanded sampling efforts.

A large body of research on estuarine fishes focuses on the roles of estuaries as nursery habitats, the value of protecting specific critical habitats, and descriptions of patterns of juvenile survival and growth. Estuaries are often viewed as critical habitats that support coastal fish populations (Beck et al., 2001), and nearshore habitat features, such as eelgrass beds, are commonly identified as key features of estuarine habitats (Levin and Stunz, 2005). Although loss of eelgrass beds has been identified as a threat in Puget Sound, their importance to the groundfish species examined here is unknown. In well-studied estuarine ecosystems, extensive time series of fish abundance indices have permitted exploration of the roles of density dependence, overwinter survival, predation, and growth-dependent mortality on year-class strength of fishes (Hurst and Conover, 1998; Buckel et al., 1999; Kim-

Table 3

Comparison of 3 models for changes in environmental time series data collected at 2 monitoring sites—West Point and Jefferson Head—in Port Madison, Puget Sound, Washington, near sampling sites at which bottom trawl surveys were conducted from 1991 to 2012. Akaike's information criteria adjusted for small sample size (AIC_c) was used to choose between the models: no change ("constant"), linear change ("linear"); or abrupt change ("change point"). Separate models were run for each monitoring site.

	Constant	Linear	Change point
Temperature			
West Point	0.00	0.62	2.85
Jefferson Head	0.00	2.41	2.85
Surface salinity			
West Point	0.00	2.41	2.85
Jefferson Head	7.89	0.00	10.81

merer et al., 2000; Taylor et al., 2009). The processes that regulate juvenile survivorship and fish population dynamics in Puget Sound are not easily discerned because of a paucity of long-term monitoring data.

Conclusions

Catch rates of resident groundfishes from a study area in Puget Sound indicated that a synchronous and abrupt decline in densities occurred in the late 1990s, counter to expectations formed on the basis of the cessation of commercial bottom trawling that preceded our sampling. Available evidence suggests that these declines may have resulted from a distributional shift rather than a demographic shift, although an analysis of data sets that span a spatial extent wider than our study area in Port Madison is needed to test this hypothesis. Therefore, considerable additional analyses are needed to address the response of species and food webs to fishing and to determine how localized closures, such as marine protected areas, may promote recovery of species. Further, there is a need to relate density shifts to environmental and biological changes (e.g., climatic drivers, human-induced habitat shifts, or trophodynamics). Finally, the unexpected shifts in localized catch rates in this study indicate a need for caution when time series are used in evaluating long-term shifts in population and community structure without consideration of whether the data are representative of entire populations.

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Appendix table

Complete list of species or taxonomic groups collected in Port Madison, Puget Sound, Washington during the period of 1991–2001, with total number of specimens collected, length range, and number of years that each was collected. * indicates that the maximum length could not be determined because of ambiguous identification of morphologically similar species. All lengths are fork length, except for Spotted Ratfish (precaudal length was measured for this species).

Species	Total number	Min. length (mm)	Max. length (mm)	Number of years
English Sole (*Parophrys vetulus*)	10,427	11	470	20
Spotted Ratfish (*Hydrolagus colliei*)	4067	80	550	20
Blackbelly Eelpout (*Lycodes pacificus*)	3398	33	997	20
Pacific Tomcod (*Microgadus proximus*)	2677	16	480	20
Slender Sole (*Lyopsetta exilis*)	2242	20	340	20
Flathead Sole (*Hippoglossoides elassodon*)	1665	60	342	20
Shiner Perch (*Cymatogaster aggregata*)	1139	10	*	16
Pacific Hake (*Merluccius productus*)	958	69	565	19
Speckled Sanddab (*Citharichthys stigmaeus*)	853	55	340	19
Rock soles (*Lepidopsetta bilineata* and *L. polyxystra*)	773	74	415	20
Sand Sole (*Psettichthys melanostictus*)	680	76	450	20
Plainfin Midshipman (*Porichthys notatus*)	497	58	326	17
Pacific Herring (*Clupea pallasii*)	478	75	320	17
Dover Sole (*Microstomus pacificus*)	366	45	381	19
Pacific Sanddab (*Citharichthys sordidus*)	222	48	352	18
Walleye Pollock (*Theragra chalcogramma*)	174	22	490	8
Pile Perch (*Rhacochilus vacca*)	170	95	211	12
Rex Sole (*Glyptocephalus zachirus*)	142	50	250	16
Snake Prickleback (*Lumpenus sagitta*)	99	50	395	16
Roughback Sculpin (*Chitonotus pugetensis*)	89	50	145	14
Shortfin Eelpout (*Lycodes brevipes*)	86	29	80	10
Pacific Staghorn Sculpin (*Leptocottus armatus*)	60	73	258	15
Tubesnout (*Aulorhynchus flavidus*)	33	64	165	11

Appendix table (cont.)

Complete list of species or taxonomic groups collected in Port Madison, Puget Sound, Washington during the period of 1991–2001, with total number of specimens collected, length range, and number of years that each was collected. * indicates that the maximum length could not be determined because of ambiguous identification of morphologically similar species. All lengths are fork length, except for Spotted Ratfish (precaudal length was measured for this species).

Species	Total number	Min. length (mm)	Max. length (mm)	Number of years
Brown Rockfish (*Sebastes auriculatus*)	33	65	325	12
C-O Sole (*Pleuronichthys coenosus*)	29	110	305	10
Slim Sculpin (*Radulinus asprellus*)	22	80	160	9
Starry Flounder (*Platichthys stellatus*)	19	110	410	12
Northern Anchovy (*Engraulis mordax*)	19	73	190	8
North Pacific Spiny Dogfish (*Squalus suckleyi*)	12	290	460	3
Bay Goby (*Lepidogobius lepidus*)	9	59	100	7
Sturgeon Poacher (*Podothecus accipenserinus*)	8	75	166	5
Longnose Skate (*Raja rhina*)	8	365	640	7
Copper Rockfish (*Sebastes caurinus*)	8	56	300	2
Sablefish (*Anoplopoma fimbria*)	6	320	400	1
Northern Spearnose Poacher (*Agonopsis vulsa*)	6	87	174	4
Eulachon (*Thaleichthys pacificus*)	6	89	150	4
Big Skate (*Raja binoculata*)	6	125	1050	3
Soft Sculpin (*Psychrolutes sigalutes*)	5	25	45	3
Snailfishes (family Liparidae)	6	24	35	5
Chinook Salmon (*Oncorhynchus tshawytscha*)	5	78	96	2
Blacktip Poacher (*Xeneretmus latifrons*)	5	140	184	1
Pacific Sand Lance (*Ammodytes hexapterus*)	4	59	145	2
Butter Sole (*Isopsetta isolepis*)	4	75	258	3
Sailfin Sculpin (*Nautichthys oculofasciatus*)	3	120	121	3
Quillback Rockfish (*Sebastes maliger*)	3	82	230	3
Curlfin Sole (*Pleuronichthys decurrens*)	3	161	260	2
Red Brotula (*Brosmophycis marginata*)	3	220	305	1
Bigfin Eelpout (*Lycodes cortezianus*)	3	260	280	1
Surf Smelt (*Hypomesus pretiosus*)	2	86	125	2
Pacific Pompano (*Peprilus simillimus*)	2	110	149	2
Pacific Cod (*Gadus macrocephalus*)	2	420	610	2
Great Sculpin (*Myoxocephalus polyacanthocephalus*)	2	220	395	2
Tadpole Sculpin (*Psychrolutes paradoxus*)	1	30	30	1
Pygmy Poacher (*Odontopyxis trispinosa*)	1	161	161	1
Pink Salmon (*Oncorhynchus gorbuscha*)	1	66	66	1
Padded Sculpin (*Artedius fenestralis*)	1	125	125	1
Northern Ronquil (*Ronquilus jordani*)	1	165	165	1
Longspine Combfish (*Zaniolepis latipinnis*)	1	230	230	1
Cabezon (*Scorpaenichthys marmoratus*)	1	410	410	1
Brown Irish Lord (*Hemilepidotus spinosus*)	1	90	90	1
American Shad (*Alosa sapidissima*)	1	274	274	1

Influence of soak time and fish accumulation on catches of reef fishes in a multispecies trap survey

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Abstract—Catch rates from fishery-independent surveys often are assumed to vary in proportion to the actual abundance of a population, but this approach assumes that the catchability coefficient (q) is constant. When fish accumulate in a gear, the rate at which the gear catches fish can decline, and, as a result, catch asymptotes and q declines with longer fishing times. We used data from long-term trap surveys (1990–2011) in the southeastern U.S. Atlantic to determine whether traps saturated for 8 reef fish species because of the amount of time traps soaked or the level of fish accumulation (the total number of individuals of all fish species caught in a trap). We used a delta-generalized-additive model to relate the catch of each species to a variety of predictor variables to determine how catch was influenced by soak time and fish accumulation after accounting for variability in catch due to the other predictor variables in the model. We found evidence of trap saturation for all 8 reef fish species examined. Traps became saturated for most species across the range of soak times examined, but trap saturation occurred for 3 fish species because of fish accumulation levels in the trap. Our results indicate that, to infer relative abundance levels from catch data, future studies should standardize catch or catch rates with nonlinear regression models that incorporate soak time, fish accumulation, and any other predictor variable that may ultimately influence catch. Determination of the exact mechanisms that cause trap saturation is a critical need for accurate stock assessment, and our results indicate that these mechanisms may vary considerably among species.

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Robust fishery-independent survey data are a critical component of modern fisheries stock assessments (Pennington and Stromme, 1998). Catch rates from fishery-independent surveys often are assumed to vary in proportion to the actual abundance of a fish population and, therefore, provide a relative measure of annual changes in abundance that can be used as a tuning index in a stock assessment (Kimura and Somerton, 2006). The basic assumption of this approach is that the catchability coefficient (q), or the efficiency of a fishery or survey gear, is constant over space, time, and over the range of environmental conditions encountered in a survey (Hilborn and Walters, 1992).

It is also typically assumed that q is not influenced by the amount of time a particular fishing gear is fished (Hamley, 1975). When the rate at which a fishing gear catches fish declines as fish accumulate in it, the fishing gear becomes saturated and q declines as fishing times increase (Miller, 1979; Olin et al., 2004). Therefore, catch rates tend to increase asymptotically rather than proportionally with fish abundance, and at high levels of abundance, catch rates are an insensitive indicator of change (Ricker, 1975). Numerous mechanisms have been shown to cause gear saturation, which can be broadly categorized as space limitation of gear, increased gear avoidance, interspecific competition, bait degradation or consumption of bait, or fishing gear that causes local depletion of fish (Kennedy, 1951; Richards et al., 1983; Olin et al., 2004). Depending on the exact mechanism that causes gear saturation, the catch at which a fishing gear becomes saturated may or may not reflect actual abundance (Beverton and Holt, 1954).

Although saturation in gill nets, longlines, and trawl nets has been well studied (Ragonese et al., 2001; Olin et al., 2004; Rodgveller et al., 2008), there has been a paucity of empirical research on the presence of trap saturation. Traps are widely used, especially in sensitive habi-

tats (e.g., seagrass meadows and coral reefs), and are sometimes the only feasible method of sampling in these habitats because of their relatively low effect on substrate and benthic communities (Miller, 1990). Clearly, the catch of fishes or invertebrates in traps cannot continue to increase linearly with soak time because the space inside a trap is finite and will eventually become filled with animals to the point at which no additional individuals can enter (Bennett, 1974; Austin, 1977; Miller, 1990). Models have been developed to describe the relationship between catch per trap and soak time, with the intention of using those models to standardize catch for different soak times (Munro, 1974; Somerton and Merritt, 1986; Zhou and Shirley, 1997). Unfortunately, these approaches to standardization do not account for landscape (e.g., depth) or environmental effects (e.g., water temperature) on catch. Nor do they help us understand how the catch of one species may be influenced by the catches of other species.

One recent method to examine these drawbacks has been to model the catch of a species as a function of soak time and fish accumulation (i.e., the total number of individuals of all species caught by a fishing gear; Olin et al., 2004) after accounting for other variables that may influence catch. In other words, catch rates can first be standardized by all of the predictor variables in the model building process (Lo et al., 1992; Maunder and Punt, 2004), and then the specific effects of soak time and fish accumulation can be extracted and examined independently of other predictor variables (Li et al., 2011). For example, Li et al. (2011) showed that gill nets became saturated with Yellow Perch (*Perca flavescens*) because of the total number of individuals caught in a gill net but not because of increased soak times. These results indicate that Yellow Perch catch rates decline when, for instance, this species sees fish already caught in the gill net, and not for reasons associated purely with increased soak time (i.e., when all the Yellow Perch in an area are caught, which takes some time).

In our study, we used a standardized catch approach to examine the influence of soak time and fish accumulation on the catches of several reef fish species from long-term fishery-independent, multispecies trap surveys occurring in the southeastern U.S. Atlantic (SEUS) from North Carolina to Florida. The inclusion of soak time and fish accumulation separated mechanisms that cause gear saturation into 2 groups: those mechanisms related to fish accumulation (e.g., agonistic behaviors or bait depletion) and those mechanisms related to the length of time a trap soaks (e.g., local depletion of the target species or loss of bait freshness). We developed a delta-generalized-additive model (delta-GAM) that was able to accommodate both nonlinearities between the response and predictor variables and zero-inflation (i.e., a high proportion of zero catches; Martin et al., 2005). This approach allowed us to determine whether trap saturation occured because of either soak time or fish accumulation, or both, and then we used the model to predict relative abundance after accounting for the influence of soak time and fish accumulation.

Materials and methods

Study area

In our study reef fish species associated with hard substrates were sampled on the continental shelf and continental shelf-break in the SEUS. The continental shelf and shelf-break in the SEUS are dominated by sand and mud substrates, within which areas of hard, rocky substrates ("hard bottom") occur and a highly diverse reef fish assemblage associates. Hard bottom habitats range in complexity from flat limestone pavement, sometimes covered with a sand or gravel veneer, to high-relief rocky ledges (Schobernd and Sedberry, 2009; Glasgow, 2010). Hard bottom areas often host diverse epifauna that can provide food and shelter for reef fishes. The major oceanographic feature of the SEUS is the Gulf Stream, which influences outer sections of the continental shelf as it flows northward. Consistently warm Gulf Stream waters along the outer SEUS shelf allow tropical and subtropical species to inhabit areas at least as far north as North Carolina (Miller and Richards, 1980). For our study, sampling occurred on continental shelf and shelf break habitats from approximately Cape Lookout, North Carolina, to St. Lucie Inlet, Florida (Fig. 1).

Sampling approach

The Marine Resources Monitoring, Assessment, and Prediction (MARMAP) Program of the South Carolina Department of Natural Resources has used chevron fish traps to index reef fish abundance since the late 1980s. Since 2009, MARMAP funding for reef fish sampling has been supplemented by the cooperative Southeast Area Monitoring and Assessment Program—South Atlantic (SEAMAP-SA) administered by the National Marine Fisheries Service. We analyzed MARMAP data from 1990 through 2011, during which time sampling with chevron fish traps was conducted in a consistent manner (as described later in this section). We also included in our analyses 2010–11 data from the Southeast Fishery-independent Survey (SEFIS), which the National Marine Fisheries Service created in 2010 to increase fishery-independent sampling in the SEUS, because sampling methods were identical. Hereafter, the 2 sampling programs are referred to as "MARMAP/SEFIS."

Hard bottom sampling stations included in the analyses were selected for sampling in 1 of 3 ways. First, most sites were selected randomly from the MARMAP/SEFIS sampling frame that consisted of approximately 2000 sampling stations on hard bottom habitat. Second, some stations in the sampling frame were sampled op-

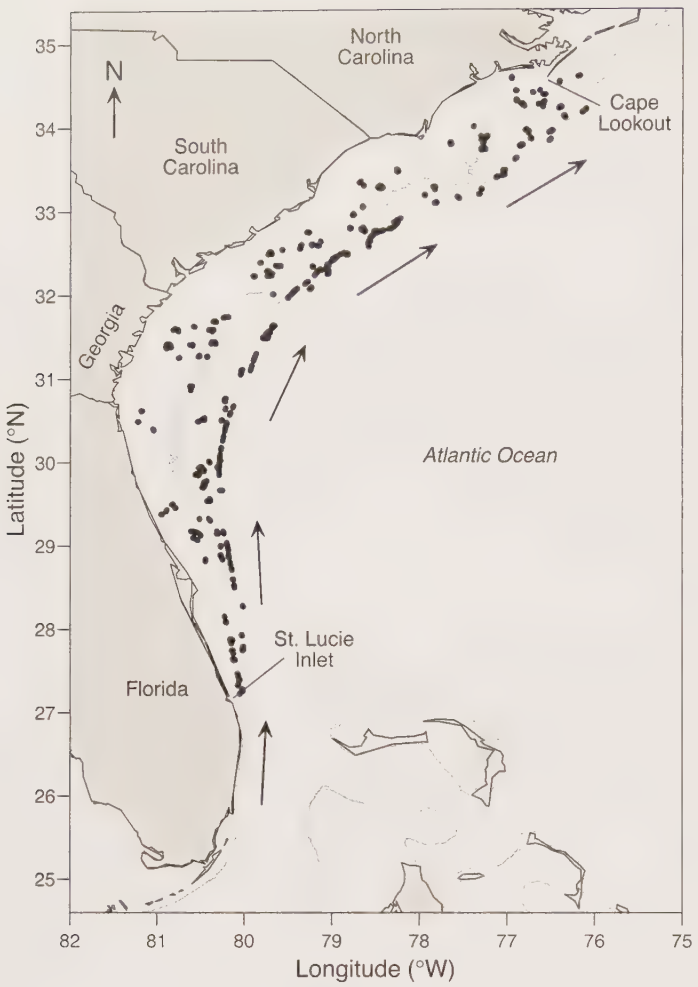

Figure 1

Spatial distribution of samples of reef fish species (black circles) collected in chevron traps during 2 long-term, fishery-independent survey programs in the southeastern U.S. Atlantic—the Marine Resources Monitoring, Assessment, and Prediction Program (1990–2011) and the Southeast Fishery-independent Survey (2010–11)—for examination of the relationship between soak time, fish accumulation, and catch of 8 reef fish species. Note that black circles overlap in many instances. Gray lines indicate 35- and 70-m depth contours (derived from bathymetry data), and arrows indicate the approximate path of the Gulf Stream.

portunistically even though they were not selected randomly for sampling in a given year. Third, new hard-bottom stations were added during the study period through the use of information from fishermen, charts, and historical surveys. These new locations were investigated with a vessel echosounder or drop cameras and sampled if hard bottom habitat was present. We assumed that the catch of each species was influenced similarly at all stations by the various predictor variables (described later). All sampling for this study occurred during daylight hours between March and October and was conducted on 1 of 4 vessels: MARMAP and SEAMAP-SA used the RV *Palmetto* (1990–2011), and SEFIS used the RV *Savannah* (2010–11), NOAA Ship *Nancy Foster* (2010), and NOAA Ship *Pisces* (2011).

Chevron fish traps were deployed at each station sampled in this study. Chevron traps were constructed from plastic-coated, galvanized 2-mm-diameter wire (mesh size=3.4 cm^2) and shaped like an arrowhead that measured 1.7 m × 1.5 m × 0.6 m, with a total volume of 0.91 m^3 (Fig. 2) (Collins, 1990). The mouth openings of traps were shaped like a teardrop and measured approximately 18 cm wide and 45 cm high. Each trap was baited with 24 menhaden (*Brevoortia* spp.): 16 were attached to freely accessible stringers and 8 were placed loosely inside. Traps typically were deployed in a group of 6. The minimum distance between individual traps was approximately 200 m to provide some measure of independence between traps.

Because the primary purpose of MARMAP/SEFIS sampling was to provide standardized catch information for reef fish species in the SEUS, a soak time of 90 min was targeted for each trap. We were not able to soak traps for a wide range of experimentally chosen amounts of time. However, for many different reasons, soak time was somewhat variable, ranging from 9 to 270 min (mean: 97.6 min and standard deviation of the mean [SD]=12.8) (Fig. 3). All trap deployments that did not fish properly (e.g., traps that dragged in current) were excluded from analysis. Soak times were variable enough to allow an examination of the ways in which fish catch was related to variability in soak time.

We included in our analyses the 8 most commonly caught reef fish species in the MARMAP/SEFIS trap surveys: Black Sea Bass (*Centropristis striata*), Tomtate (*Haemulon aurolineatum*), Red Porgy (*Pagrus pagrus*), Bank Sea Bass (*Centropristis ocyurus*), Gray Triggerfish (*Balistes capriscus*), Vermilion Snapper (*Rhomboplites aurorubens*), *Stenotomus* spp., and Sand Perch (*Diplectrum formosum*) (Table 1). *Stenotomus* spp. may represent more than one species, but, for the purpose of discussion, we will refer to this taxon as a single species and the group of taxa studied as 8 species of fish. Fish coloration, shape, and meristics were used to identify individuals to genus and species levels with field guides (e.g., Robins et al., 1986; Hoese and Moore, 1998; McEachran and Fechhelm, 1998; Carpenter, 2002; Humann and Deloach, 2002; McEachran and Fechhelm, 2005). Black Sea Bass, Red Porgy, Gray Triggerfish, and Vermilion Snapper are targeted by commercial and recreational fishermen in the SEUS. The 8 species included in our analyses were the most common species caught in the traps in the MARMAP/SEFIS surveys by both frequency of occurrence and mean catch per trap (Table 2). Additional species were not analyzed if their frequency of occurrence was less

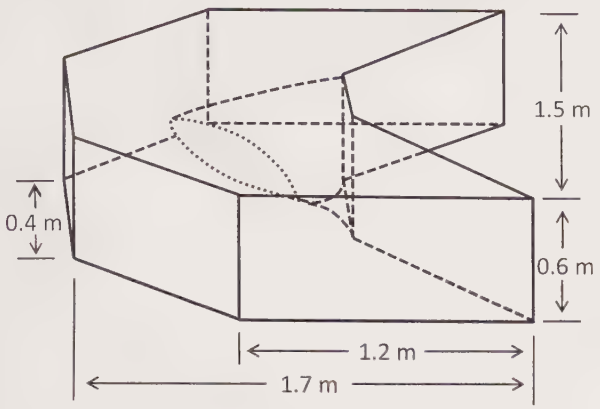

Figure 2

Schematic of the design of the chevron trap used to sample reef fish species in 1990–2011 by the Marine Resources Monitoring, Assessment, and Prediction Program and the Southeast Fishery-independent Survey. The gray oval is the mouth opening of the trap (~18 cm wide and 45 cm high), and the total trap volume is 0.91 m^3.

than 15%, but individuals of all species were included in analyses of fish accumulation.

Data analyses

We used a generalized additive modeling approach to test the hypothesis that trap catch of reef fishes was influenced by soak time and fish accumulation. GAMs use nonparametric smoothing functions to account for nonlinearities between predictor and response variables (Hastie and Tibshirani, 1990; Bacheler et al., 2009). GAMs extend traditional additive models by allowing for alternative distributions of underlying random variation, just as generalized linear models allow for alternative distributions in linear models.

We developed delta-GAMs because there was a high proportion of zero observations (>50%) for the catch of each species that could not be modeled appropriately with standard statistical distributions. We considered zero-inflated models, but they were challenging to work with because they rarely converged, and when they did, model solutions were often unreasonable. Therefore, we developed a delta-GAM for each of the 8 species. Each delta-GAM contained 2 submodels that estimated the influence of soak time and fish accumulation on catch:

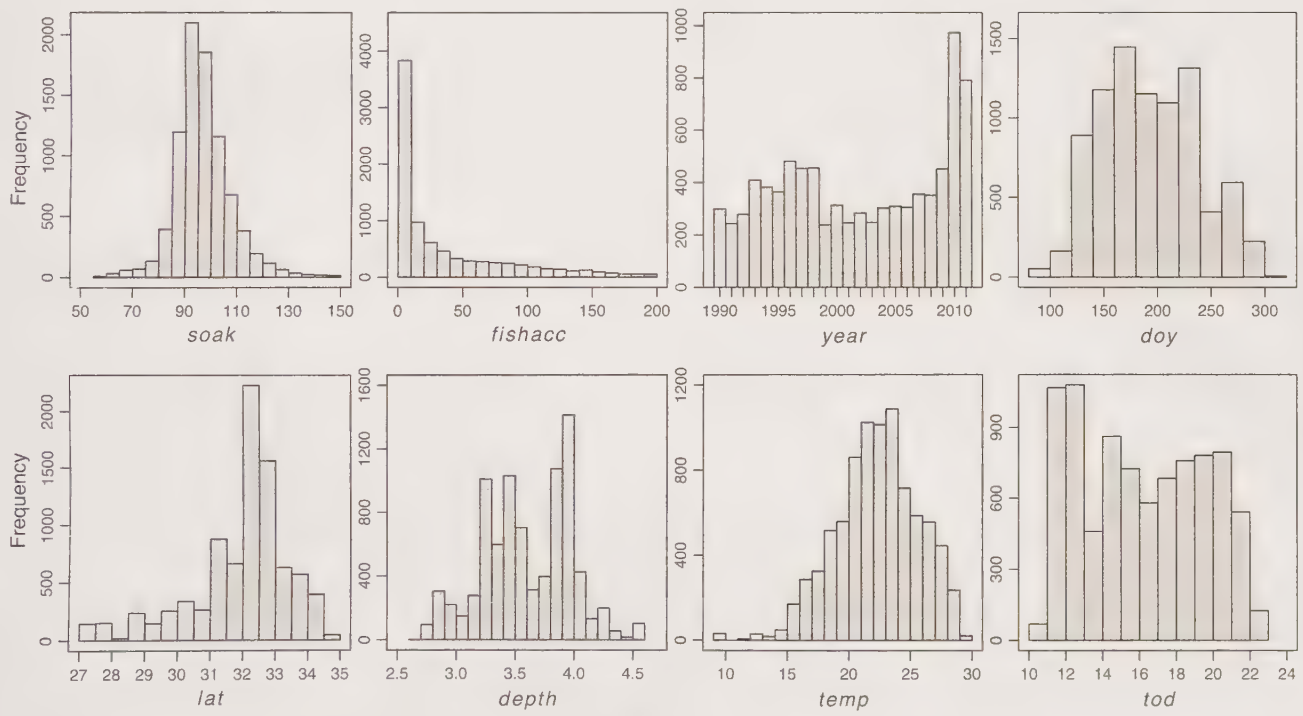

Figure 3

Histograms of predictor variables used in the delta-generalized-additive models of reef fish catch from trap surveys conducted by the Marine Resources Monitoring, Assessment, and Prediction Program and the Southeast Fishery-independent Survey in the Atlantic Ocean from North Carolina to Florida during 1990–2011. Variables were soak time (*soak*; min), fish accumulation (*fishacc*; total fish per trap), year of sampling (*year*), day of the year (*doy*), latitude (*lat*; °N), depth of sampling (*depth*; m), bottom water temperature (*temp*; °C), and time of day (*tod*; Coordinated Universal Time).

Table 1

Life history characteristics of the 8 fish species analyzed in our study of soak time and fish accumulation as mechanisms that can cause trap saturation. Characteristics come from data sets of 2 sampling programs in the southeastern U.S. Atlantic: the Marine Resources Monitoring, Assessment, and Prediction Program (1990–2011) and the Southeast Fishery-independent Survey (2010–11). L_{∞}=maximum length; $L_{maturity}$=length at maturity; A_{max}=maximum age in years; f=fish; i=invertebrates; PH=protogynous hermaprodite; SH=sequential hermaphrodite; GO=gonochoristic. All lengths are total lengths in centimeters.

Common name	Scientific name	L_{∞}	$L_{maturity}$	A_{max}	Diet	Reproduction
Bank Sea Bass	*Centropristis ocyurus*	33[a]	14[a]	9[a]	f,i[a]	PH[a]
Black Sea Bass	*Centropristis striata*	50[a]	16[a]	11[a]	f,i[b]	PH[a]
Gray Triggerfish	*Balistes capriscus*	48[a]	19[a]	12[a]	i[f]	GO[a]
Red Porgy	*Pagrus pagrus*	51[a]	25[a]	20[a]	f,i[e]	PH[a]
Sand Perch	*Diplectrum formosum*	24[k]	19[k]	8[k]	f,i[l]	SH[a]
Tomtate	*Haemulon aurolineatum*	35[c]	18[a]	17[a]	i[d]	GO[a]
Vermilion Snapper	*Rhomboplites aurorubens*	51[a]	15[g]	13[a]	f,i[h]	GO[a]
	Stenotomus spp.	46[l]	18[i]	15[j]	i[l]	GO[a]

Sources: [a] MARMAP (unpubl. data); [b] Sedberry (1988); [c] Manooch and Barans (1982); [d] Sedberry (1985); [e] Manooch (1977); [f] Kurz (1995); [g] Zhao et al. (1997); [h] Sedberry and Cuellar (1993); [i] O'Brien et al. (1993); [j] Finkelstein (1969); [k] Bubley and Pashuk (2010); [l] South Atlantic Fishery Management Council (http://www.safmc.net/).

one modeling the presence-absence of each species and another modeling the positive catches only (Lo et al., 1992; Pennington, 1996; Stefansson, 1996). The overall effects of a particular predictor variable on catch were then obtained by multiplying the effects from each submodel (Maunder and Punt, 2004; Murray, 2004; Li et al., 2011).

We examined the influence of 8 predictor variables on the catch of 8 reef fish species: soak time, fish accumulation, year, depth, time of day, day of the year, water temperature, and latitude (Fig. 3). In this study, we were particularly interested in how soak time and fish accumulation influenced the catch of each species after accounting for variability in the other 6 predictor variables. To accomplish this goal, we first fitted the delta-GAM, and then we predicted catch for a range of values for soak time and fish accumulation, fixing all other predictor variables to their own mean, except the variable *year*, which was fixed at year 2000 (i.e., the midpoint of the time series; results were invariant to the year chosen). Soak time (*soak*) was the number of minutes a trap soaked between deployment and retrieval, and fish accumulation (*fishacc*) was the total number of individuals of all fish species caught

Table 2

Catch information, mean length, and frequency of occurrence (FO) for each of the 8 most common species of reef fishes in the data sets of 2 sampling programs in the southeastern U.S. Atlantic: the Marine Resources Monitoring, Assessment, and Prediction Program (1990–2011) and the Southeast Fishery-independent Survey (2010–11). Mean proportion of catch is the mean proportion of catch in each trap that was composed of a single species. Fork length was measured for all species, except Black Sea Bass (*Centropristis striata*) and Bank Sea Bass (*C. ocyurus*), which were measured for total length. SD=standard deviation of the mean.

Species	FO	Mean (SD) catch per trap	Mean (SD) proportion of catch	Mean (SD) length (cm)
Bank Sea Bass	0.301	1.4 (3.9)	0.06 (0.17)	22.5 (3.0)
Black Sea Bass	0.408	10.2 (21.8)	0.20 (0.30)	23.6 (4.5)
Gray Triggerfish	0.263	0.9 (3.3)	0.06 (0.18)	31.3 (7.0)
Red Porgy	0.364	2.0 (4.4)	0.13 (0.26)	27.1 (4.6)
Sand Perch	0.184	0.6 (1.8)	0.03 (0.13)	22.8 (1.6)
Stenotomus spp.	0.203	6.5 (19.8)	0.09 (0.20)	15.6 (2.0)
Tomtate	0.403	9.3 (21.9)	0.18 (0.28)	18.1 (1.9)
Vermilion Snapper	0.263	3.0 (9.9)	0.07 (0.17)	23.7 (3.9)

in a trap. We limited our analysis to *soak* between 50 and 150 min and to *fishacc* less than 200 total fish per trap because of low small sample sizes outside of these ranges (i.e., <2% of all observations). *Year* was included as a categorical variable (1990–2011), but all other variables were included as continuous variables. Depth (*depth*) was measured in meters for each trap deployment; depths were recorded in a range of 13–218 m over the course of the surveys used in our study. Because of small sample sizes, samples collected at depths >100 m were excluded from our analyses and remaining depths were log-transformed to achieve normality. Time of day (*tod*) was measured in Coordinated Universal Time, and day of the year (*doy*) was the day of the year that the trap sample was collected. Water temperature (*temp*) was bottom water temperature measured in degrees Celsius for each group of 6 simultaneously deployed traps, and latitude (*lat*) was the latitude (degrees north) at which the samples were collected. Longitude was not included because of its statistically significant correlation ($P<0.001$) with depth that occurred because of the north–south orientation of our study area.

Before the development of models, multicollinearity among predictor variables was examined because its presence can cause erratic model behavior and should be avoided (Zar, 1999). We assessed the severity of multicollinearity among predictor variables through calculation of the variance inflation factor (VIF) for each variable, which measures the amount of variance that is inflated for each variable as a result of its collinearity with other predictor variables. The VIF for all predictor variables was less than 4.0—below the level generally acknowledged to be problematic (5–10; Neter et al., 1989)—thus indicating no significant multicollinearity among predictor variables in our data set.

We also included *fishacc* as a predictor variable in our GAMs. Because trap catch often was composed of a mixture of fish species in the multispecies survey, the contribution of a single species to the *fishacc* variable was generally small, but there were instances when the catch in traps was dominated by a single species. Inclusion of samples in which the catch was dominated by a single species may have positively biased the reported deviance of the models for those particular species, but the functional relationship between single species catch and *fishacc* was not affected. If catch of a single species was influenced entirely by *fishacc*, the relationship between the 2 variables would have been perfectly linear. By definition, then, any deviation from a linear relationship between the 2 variables could not have been the result of a potential lack of independence. Because we were primarily interested in the shape of the relationship between catch and *fishacc*, we agree with Li et al. (2011) that the inclusion of the *fishacc* variable is a useful approach to examine trap saturation due to fish accumulation.

Initially, a full model was fitted on the presence-absence data for each species. Following Li et al. (2011), we used a binomial GAM submodel to estimate the probability of presence for each species being caught in individual traps (η), which was assumed to be an independent draw from a binary variable with a probability of success p:

$$E(p) = \upsilon^{-1}(\eta); \tag{1}$$

$$\eta = \alpha + g_1(soak) + g_2(fishacc) + \sum_{j=3}^{N} g_j(x_j),$$

where $E(p)$ = the expectation of p;
υ = the logit link function;
α = the intercept;
soak = soak time;
fishacc = fish accumulation;
g_j are smoothing functions,
N = the number of predictor variables in the model; and
x_j = the jth remaining explanatory variable.

We next coded a positive-catch GAM submodel that related the Gaussian fourth-root transformed catch of each reef fish species when caught to the 8 predictor variables. We compared the error structure of log-normal, log-gamma, and Gaussian (with a fourth-root transformation) distributions using the Akaike information criterion (AIC; Burnham and Anderson, 2002) for each full model:

$$\mathrm{AIC} = -2\log\left(\mathcal{L}(\hat{\theta} \mid y)\right) + 2K, \tag{2}$$

where $\left(\mathcal{L}(\hat{\theta} \mid y)\right)$ = the log-likelihood; and
K = the number of parameters of each model.

The model with the lowest AIC value was considered the best model in the model set. For all species, the Gaussian distributions with fourth-root transformations had the lowest AIC values and were therefore considered the most parsimonious distributions for the positive-catch submodels.

For the positive-catch GAM submodel, we used the following equation:

$$y^{0.25} = a' + h_1(soak) + h_2(fishacc) + \sum_{j=3}^{N} h_j(x_j), \tag{3}$$

where y = the trap catch of a particular reef fish species;
a' = the intercept;
h_j are smoothing functions;
N = the number of predictor variables in the model; and
x_j = the jth remaining explanatory variable.

For each reef fish species, we then compared the full GAM submodels containing 8 predictor variables to various reduced models that contained fewer predictor variables. We compared various binomial GAM submodels with the unbiased risk estimator (UBRE) score,

Table 3

Final generalized additive models (GAMs) for 8 species of reef fishes in our study. Data were obtained from 2 sampling programs in the southeastern U.S. Atlantic: the Marine Resources Monitoring, Assessment, and Prediction Program (1990–2011) and the Southeast Fishery-independent Survey (2010–11). Binomial GAMs were constructed with presence-absence data, and Gaussian GAMs were constructed with only positive catch. The best model for each species was the one with the lowest unbiased risk estimator (binomial GAM) or generalized cross validation (Gaussian GAM) scores (see the *Materials and methods* section for full descriptions). *N*=number of samples from chevron traps that were included in each model. Dev. exp.= the percentage of deviance explained by each model, *ex* means that the covariate was excluded from the final model, g_1... g_7 are nonparametric smoothing functions, *f*=a categorical function, *soak*=soak time, *fishacc*=fish accumulation, *year*=year, *doy*=day of the year, *lat*=latitude, *depth*=bottom depth, *temp* =bottom temperature, and *tod*=time of the day. Estimated degrees of freedom and statistical significance are shown for each term: *=$P \leq 0.10$, **=$P \leq 0.05$, ***=$P \leq 0.01$.

Model and species	*N*	Dev. exp.	g_1(*soak*)	g_2(*fishacc*)	f_1(*year*)	g_3(*doy*)	g_4(*lat*)	g_5(*depth*)	g_6(*temp*)	g_7(*tod*)
Binomial GAM										
Bank Sea Bass	8530	28.8	ex	3.0***	21***	8.6***	8.1***	8.9***	6.3***	8.3***
Black Sea Bass	8530	63.6	ex	3.0***	21***	2.6***	8.6***	8.2***	8.1***	1.0**
Gray Triggerfish	8530	20.6	5.5***	3.0***	21***	8.9***	8.8***	5.8***	4.8***	8.1**
Red Porgy	8530	27.0	1.6*	3.0***	21***	8.0***	8.9***	7.8***	8.5***	5.1**
Sand Perch	8530	37.5	1.7***	6.8***	21***	ex	7.0***	7.8***	6.1	1.0***
Stenotomus spp.	8530	61.4	3.3***	7.4***	21***	ex	5.9***	6.0***	7.9***	8.1***
Tomtate	8530	46.9	5.6***	8.2***	21***	2.8***	8.9***	8.3***	7.1***	2.0*
Vermilion Snapper	8530	38.0	1.6	8.6***	21***	1.0***	8.2***	7.1***	7.1***	8.2***
Gaussian GAM										
Bank Sea Bass	2571	22.1	ex	2.9***	21***	8.9***	8.2***	8.5***	6.8***	3.9***
Black Sea Bass	3476	64.4	ex	8.1***	21***	7.9***	8.6***	7.2***	1.6***	4.8***
Gray Triggerfish	2244	18.9	2.3**	2.9***	21***	1.0***	7.5***	7.4***	1.0	7.7***
Red Porgy	3104	21.4	3.4*	2.0***	21***	2.1	8.8***	8.8***	2.2	1.0**
Sand Perch	1568	26.5	ex	2.8***	21***	5.3	8.9***	2.7***	3.4*	ex
Stenotomus spp.	1733	48.6	4.0*	4.0***	21***	1.0	8.8***	7.9***	5.4***	4.8**
Tomtate	3437	51.2	ex	7.0***	21***	6.2**	8.8***	8.8***	7.7***	4.4**
Vermilion Snapper	2240	36.1	ex	6.4***	21***	ex	8.0***	5.4***	1.6***	ex

which is effectively a rescaled AIC approach that is well suited for binomial models (Wahba, 1990). For the positive-catch GAM submodels, we used generalized cross validation (GCV; a measure of the out-of-sample prediction mean squared error) to select the most parsimonious combination of predictor variables. For each approach, the model for each species with the smallest UBRE or GCV score was selected as the best model in that particular model set. In addition, we evaluated the model diagnostics for each final model selected by UBRE or GCV. In all cases, residuals in final models met assumptions of normality and constant variance. All models were coded and analyzed in R,[1] vers. 2.14.1 (R Development Core Team, 2011) with the mgcv library, vers. 1.7-13 (Wood, 2008).

We used 2 methods to test for the presence or absence of spatial autocorrelation, which is the situation where samples near one another are often more similar than 2 samples farther apart. First, we developed generalized additive mixed models (GAMMs) for each species with the same covariates as the GAM models presented previously. GAMMs are spatially explicit regression models that allow for spatially correlated error distributions (Venables and Ripley, 2002). Using positive-catch data, we found that the coefficient of multiple determination (R^2) and parameter significance values from the GAMMs were nearly identical to GAM model results for all 8 species. Binomial GAMMs built on presence-absence data never converged for any species. Second, we developed semivariograms for each species for each year using the R package geoR, vers. 1.7-2 (R Development Core Team, 2011). There were no consistent patterns in the relationship between the semivariance of the model residuals and distance between sampling points, indicating negligible spatial autocorrelation in the residuals.

The overall influence of *soak* or *fishacc* on reef fish catch was calculated as the product of the binomial and positive-catch submodels, and the variance of the overall model was estimated with a bootstrapping approach. We resampled the predictions (N=10,000) for both submodels at average values of all other predictor variables according to the pointwise estimates of error that were assumed to be distributed normally. For the combined (overall) predictions, we multiplied the simulated point estimates of error for each submodel. The

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

95% confidence interval was estimated as the 0.025 and 0.975 quantiles of the 10,000 point estimates.

Results

The assumption of independence of predictor variables was met in our study. There were no statistically significant relationships between any of the predictor variables included in our analyses (VIF<4), and, in particular, *soak* and *fishacc* variables were unrelated (r^2<0.001). *Fishacc* was the total number of individuals of all species caught in a trap, whereas the response variable (i.e., catch) was the total number of individuals of each species caught in a trap. Moreover, no single species composed more than 20% of total trap catch (Table 2).

Overall, 8530 samples collected from chevron traps in the 22-year span of MARMAP/SEFIS surveys were included in our analyses (annual mean: 388 |SD 179|). Sampling occurred between March and October, and there were no obvious changes in the seasonality of sampling across years. The spatial coverage of the surveys, in contrast, expanded southward in the mid-1990s to include sampling sites in central Florida.

Of the 8 reef fish species included in our analyses, Black Sea Bass had the highest frequency of occurrence (0.408) and Sand Perch had the lowest (0.184; Table 2). Unstandardized catch per trap ranged from 0.6 for Sand Perch to 10.2 for Black Sea Bass. Mean lengths ranged from 15.6 cm fork length for *Stenotomus* spp. to 31.3 cm fork length for Gray Triggerfish (Table 2).

The binomial GAMs explained 20.6–63.6% of the deviance in presence-absence patterns of the 8 reef fish species (Table 3). Models that explained the least deviance were the ones for Gray Triggerfish (20.6%) and Red Porgy (28.8%), and models for Black Sea Bass (63.6%) and *Stenotomus* spp. (61.4%) explained the most deviance. All 8 predictor variables were selected in the binomial model for each species on the basis of UBRE scores, with the exceptions of *soak* for Black Sea Bass and Bank Sea Bass and *doy* for *Stenotomus* spp. and Sand Perch (Table 3). The *fishacc* variable was retained in the binomial models for all species.

The Gaussian GAMs explained 18.9–64.4% of the deviance in the positive-catch values of the 8 reef fish species (Table 3). The most deviance was explained by the models for Black Sea Bass (64.4%) and Tomtate (51.2%), and models for Gray Triggerfish (18.9%) and Red Porgy (21.4%) explained the least deviance (Table 3). On the basis of GCV scores, *soak* was excluded from the models for Black Sea Bass, Bank Sea Bass, Tomtate, Vermilion Snapper, and Sand Perch. Moreover, *doy* was excluded from the Vermilion Snapper model, and *tod* was excluded from the models for Vermilion Snapper and Sand Perch. The *fishacc* variable was included in all 8 models.

Over the range of *soak* values examined, predicted overall catch was invariant to *soak* for 3 species (Black Sea Bass, Bank Sea Bass, and Vermilion Snapper), positively related to *soak* for 2 species (Red Porgy and Gray Triggerfish), and negatively related to *soak* for the remaining 3 species (Tomtate, *Stenotomus* spp., and Sand Perch; Fig. 4). For most species, the influence of *soak* on the probability of obtaining nonzero catch was very similar to its influence on the estimated overall catch when present (Fig. 4). With a doubling of *soak* from 60 to 120 minutes, estimated catch approximately doubled for Red Porgy (106% increase) and Gray Triggerfish (95%) but increased little for Black Sea Bass (20%), Bank Sea Bass (9%), and Vermilion Snapper (8%; Table 4). The estimated overall catch of Sand Perch (–71%), Tomtate (–32%), and Sand Perch (–26%) declined when *soak* doubled (Table 4). Note that confidence intervals were larger for Gray Triggerfish and *Stenotomus* spp. than for the other 6 species.

The relationship between the overall catch of the 8 reef fish species and *fishacc* displayed one of 3 patterns (Fig. 5). The overall catch of Black Sea Bass, Tomtate, Vermilion Snapper, and Sand Perch generally was related linearly to *fishacc*, indicating that the rate of catch of these species was not strongly influenced by the catch of individuals of all species in the trap. Alternatively, the overall catch of Red Porgy, Bank Sea Bass, and Gray Triggerfish reached an asymptote at *fishacc* values between 50 and 100, indicating that individuals of these 3 species were much less likely to enter a trap once 50 to 100 total individuals of all species were caught in it. The last pattern was displayed by *Stenotomus* spp., the overall catch of which was exponentially related to *fishacc*, indicating that *Stenotomus* spp. were more likely to enter a trap once large numbers of individual of all species were caught (Fig. 5). All 8 species had relatively narrow 95% confidence intervals surrounding overall mean estimates.

Discussion

Fishery-independent survey data form a critical component of modern stock assessments because they produce indices of abundance that are assumed to vary in proportion to the actual abundance of a population (Pennington and Stromme, 1998). Whether or not indices of abundance track actual abundance is a complex topic that has been the subject of much research (Harley et al., 2001; Kimura and Somerton, 2006). The basic assumption of this approach is that q does not change over space, time, or environmental conditions (Hilborn and Walters, 1992). In our study, we tested whether q was influenced by 2 additional variables, the length of time the trap was soaked and fish accumulation. For the species in our study, there was no clear relationship between life history traits and mechanisms of trap saturation (Table 1). We found evidence of trap saturation for most of the reef fish species examined, but the responses were species-specific. Trap saturation was observed for some reef fishes across the range of soak

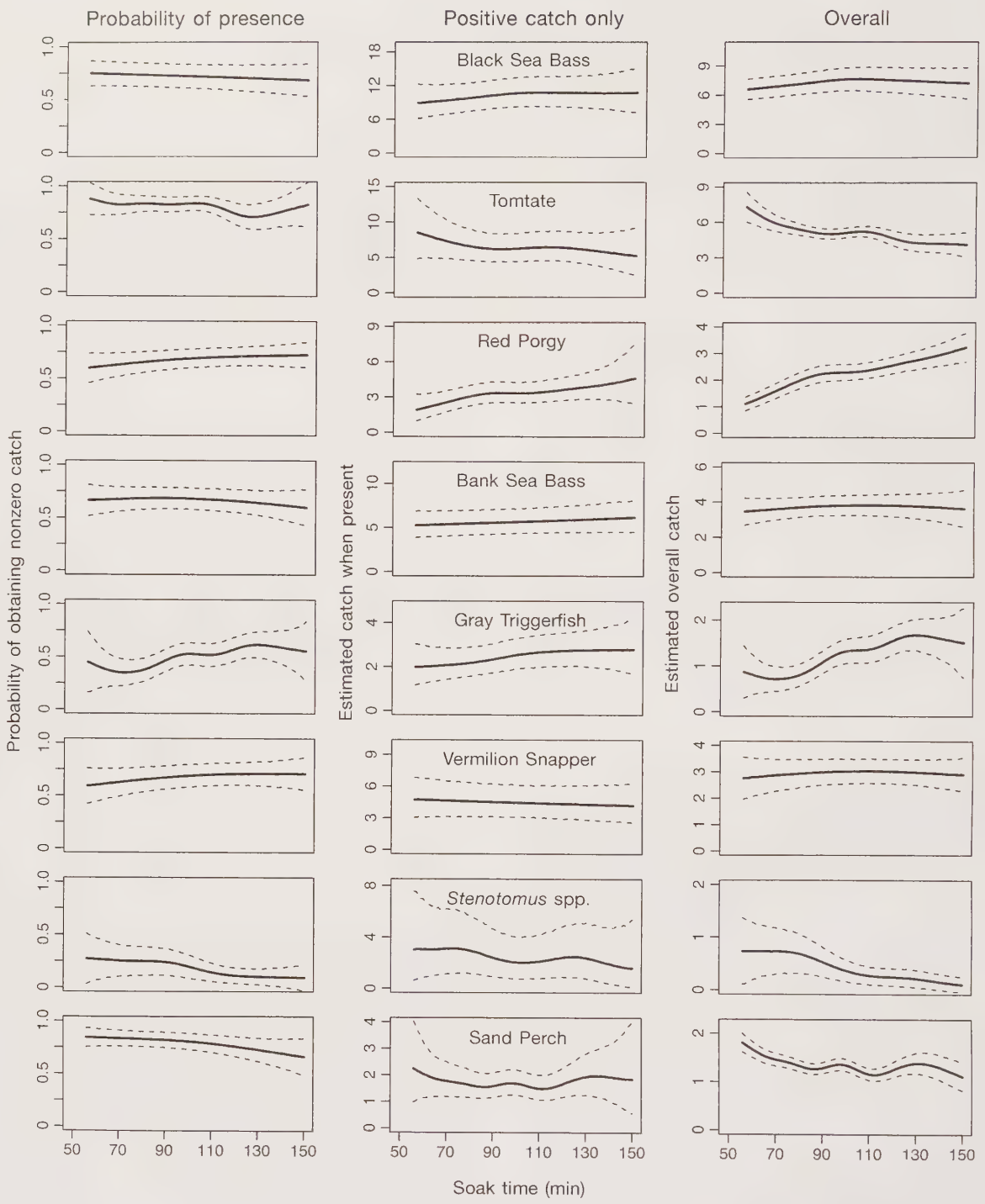

Figure 4

Relationship between soak time and the probability of obtaining a nonzero catch (probability of presence) (left column), estimated catch when present (middle column), and estimated overall catch (right column) for 8 species of reef fishes on the basis of data from trap surveys conducted in the southeastern U.S. Atlantic by the Marine Resources Monitoring, Assessment, and Prediction Program (1990–2011) and the Southeast Fishery-independent Survey (2010–11). Binomial generalized additive models (GAMs) were used to estimate probability of obtaining nonzero catch. Gaussian GAMs were used to estimate catch when present. Overall catch was determined through the combination of binomial and Gaussian GAM estimates. Dashed lines indicate 95% confidence intervals.

times examined, and, for others, because of fish accumulation in the trap.

A number of methods have been developed to determine if gear saturation is occurring. Addison and Bell (1997) used a simulation approach to show that the relationship between lobster catch and abundance was asymptotic, a problematic result because models would predict an even spatial distribution of lobster catches across a study area despite a true underlying aggregated distribution. Some researchers have documented gear saturation by the fact that the cumulative catch of individuals in traps that are periodically emptied is often much higher than the catch in traps that were hauled and redeployed without being emptied (Miller, 1979; Robertson, 1989). Alternatively, Li et al. (2011) developed a delta-GAM to quantify the relationship between catch of Walleye (*Sander vitreus*) and Yellow Perch and soak time or fish accumulation at average values of all other covariates in the model. We used the Li et al. (2011) modeling approach to show that chevron traps became saturated for all 8 reef fish species examined across a range of values for soak time or fish accumulation. There are 3 major benefits of this approach. First, it is possible to test for trap saturation through the use of long-term survey data, as long as there has been sufficient variation in soak time. Second, the relationship between catch and soak time or fish accumulation can be quantified after accounting for variation due to the other predictor variables in the model. Last, zero-inflation, the situation where a large number of zero observations in a data set cannot be properly accounted for with traditional statistical distributions, can be properly accounted for through the use of a delta model.

Table 4

Percent change in estimates of overall catch per trap for 8 reef fish species when soak time doubled (from 60 to 120 min) on the basis of data from 2 sampling programs in the southeastern U.S. Atlantic: the Marine Resources Monitoring, Assessment, and Prediction Program (1990–2011) and the Southeast Fishery-independent Survey (2010–11). Catch was estimated with a delta-generalized-additive model where mean values of all other predictor variables were used. Species are arranged from highest to lowest percent change in catch per trap.

Species	Estimated catch at 60 min	Estimated catch at 120 min	Percent change
Red Porgy	1.25	2.57	106
Gray Triggerfish	0.80	1.56	95
Black Sea Bass	6.45	7.71	20
Bank Sea Bass	3.51	3.83	9
Vermilion Snapper	2.79	3.02	8
Sand Perch	1.67	1.24	–26
Tomtate	6.78	4.58	–32
Stenotomus spp.	0.99	0.29	–71

Our study was improved by the inclusion of the *fishacc* variable in the models. If the rate at which a species entered the trap was unaffected by the number of individuals (of all species) already caught in a trap, then one would expect a positive, linear relationship between the catch of a species and the *fishacc* variable (Li et al., 2011). We showed that catches of Red Porgy, Bank Sea Bass, and Gray Triggerfish plateaued once a moderate number of total individuals were already caught in a trap, indicating that these species are more sensitive to species interactions and, therefore, much less likely to enter a trap once it began filling up. These results are consistent with previous work that has shown that behavioral interactions in and around traps can strongly influence the catch of target species (Addison and Bell, 1997; Jury et al., 2001; authors, personal observ.). In contrast and, perhaps, more surprisingly, Black Sea Bass, Tomtate, Vermilion Snapper, and Sand Perch continued to enter a trap at about the same rate no matter how many total individuals of all species were caught in it. A primary benefit of inclusion of a predictor variable for fish accumulation in our model was that it allowed us to distinguish between species that saturated the gear because of fish accumulating in a trap from the species that appeared to saturate the gear because of the amount of time a trap soaked. In addition, the inclusion of the *fishacc* variable standardized the catch of each of the 8 reef fish species to a common total catch of all species in the trap. In other words, we were able to remove variations in the catch of each reef fish species that were attributable to species-specific responses to fish accumulation. The inclusion of a variable for fish accumulation in a standardization model is one straightforward approach that can be used to account for some species interactions.

Our results indicate that catch per trap, not catch per trap minute, should be the response variable used in future standardization models for all the species we examined. If catch is invariant to soak time and catch per unit of effort (CPUE) is used as the response in a catch standardization model, then CPUE on average would be lower in traps with longer soak times than in traps with shorter soak times. Soak-time-dependent CPUE could become a serious problem if soak time for traps was longer in some years than in others because real changes in the relative abundance of a species would be confounded with the effects on CPUE due to changes in soak time. Instead, we recommend the use of a model-based approach with catch as the response variable and soak time and fish accumulation as predictor variables to properly account for any variation in catch due to these 2 factors.

Whether catch data from trap surveys can be used to index reef fish abundance ultimately depends on the underlying mechanisms responsible for the observed

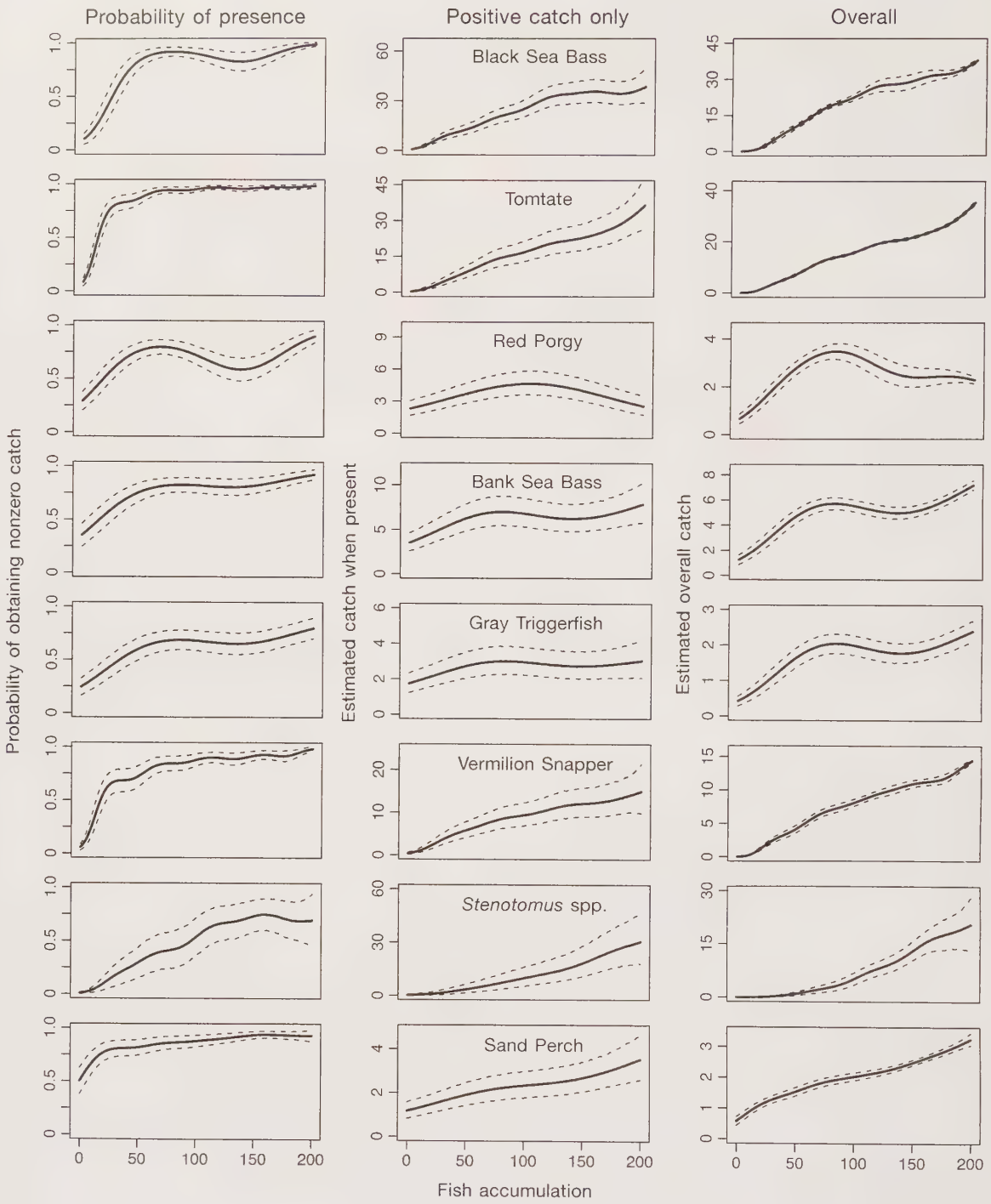

Figure 5

Relationship between fish accumulation (total fish per trap) and the probability of obtaining a nonzero catch (probability of presence) (left column), estimated catch when present (middle column), and estimated overall catch (right column) for 8 species of reef fishes on the basis of data from trap surveys conducted in the southeastern U.S. Atlantic by the Marine Resources Monitoring, Assessment, and Prediction Program (1990–2011) and the Southeast Fishery-independent Survey (2010–11). Binomial generalized additive models (GAMs) were used to estimate probability of obtaining nonzero catch. Gaussian GAMs were used to estimate catch when present. Overall catch was determined through the combination of binomial and Gaussian GAM estimates. Dashed lines indicate 95% confidence intervals.

trap saturation. For instance, if catch reaches an asymptote because most or all of the individuals in a local area have been caught, then catch is likely a good index of abundance. But catch may not reflect actual abundance if trap saturation occurs because of space limitation in the gear, negative species interactions, increasing avoidance of the gear due to individuals already being caught, handling time limitations, or bait deterioration or consumption of bait (Kennedy, 1951; Munro, 1974; Olin et al., 2004). If traps become saturated at a level of catch unrelated to actual abundance, then statistical models for censored data may be useful (Bagdonavicus et al., 2011). Another factor that may affect trap saturation is changes in feeding motivation of fishes with time of day or light levels. In our study, changes in feeding with time of day were not related to catch because all trapping was done during daylight hours. Light levels, therefore, were driven by water clarity more than by time of day. However, preliminary occupancy and *N*-mixture modeling for a few species has shown that water clarity does not appear to influence the detection probability of traps.[2] In our study, we could not identify the exact mechanisms responsible for trap saturation; therefore, this topic clearly requires more research.

Catches of Tomtate, *Stenotomus* spp., and Sand Perch declined with increasing soak times, indicating that at least some individuals of these 3 species may have escaped from the trap. These results are consistent with our own observations and a growing body of literature that indicates that some fish, crab, and lobster species frequently escape from traps and pots (Jury et al., 2001; Cole et al., 2004; Sturdivant and Clark, 2011). Tomtate, *Stenotomus* spp., and Sand Perch were among the 3 smallest fish species examined in our study, and their small size may have allowed them to escape through the narrow trap entrance more easily than could species of larger size. Size was the only life-history trait (Table 1) that influenced catch. An alternative explanation for the decreased catch of these 3 species during soak times over 100 min is that they had a longer time over which they may have been exposed to and eaten by predatory fish species caught in the same trap. We consider this explanation less likely because the diets of predatory fishes caught in traps only occasionally contain freshly consumed Tomtate and *Stenotomus* spp. (Goldman[3]), but the 2 explanations are not mutually exclusive. Experimental work should be conducted with underwater video to quantify entry and exit rates of reef fishes in fish traps—research that can provide significant insights into the catch dynamics and species interactions of marine organisms (e.g., Jury et al., 2001; Cole et al., 2004; Sturdivant and Clark, 2011).

Delta-GAMs provided a convenient analytical approach that helped us understand the influence of soak time and fish accumulation on the catch of reef fish species in a multispecies trap survey. The primary benefit of a delta-GAM is that the effects of soak time and fish accumulation can be understood after accounting for variation in total fish catch that might be due to all the other predictor variables in a model (Li et al., 2011). By accounting for soak time, fish accumulation, and other predictor variables, we found an improvement over previous (primarily gill net) studies that focused on only those predictor variables that were directly related to the gear saturation process itself (e.g., Minns and Hurley, 1988; Hansen et al., 1998; Akiyama et al., 2007). A secondary benefit of delta-GAMs is that they can account for zero-inflation. It is important to note that delta-GAMs, which are composed of separate submodels that must be combined, have been criticized as less elegant than the recently developed zero-inflated GAMs to account for zero-inflation (Chiogna and Gaetan, 2007; Liu and Chan, 2011). In our study, zero-inflated models were challenging to work with because they rarely converged, and, when they did, model solutions were often unreasonable.

There were some potential drawbacks of our experimental design and analyses. First, the range of soak times used in our study (50–150 min) was relatively narrow, and broader insights into the catch dynamics of species in traps would have been possible if large numbers of traps had been soaked for much shorter or longer periods of time. Second, the *fishacc* variable was made up partially of the catch of each individual species (Olin et al., 2004; Li et al., 2011), but we do not consider this aspect of our study to be a problem because we were interested primarily in the shape of the relationship between catch and fish accumulation, not necessarily in the significance of *fishacc* in the delta-GAMs. Third, the *fishacc* variable did not distinguish large, predatory species from smaller, nonpredatory species caught in the trap; future analyses could separate the catch of potential predators from smaller species.

Conclusions

We showed that the rate at which reef fish species entered traps in long-term programs of fishery-independent surveys decreased either over a range of soak times or over a range of fish accumulation levels. Trap saturation occurred for all 8 reef fish species that we examined; therefore, we recommend that future studies use catch standardization on raw catch or CPUE data (Maunder and Punt, 2004). It is also extremely important to understand the exact mechanisms that cause fish to saturate fishing gears, and our results indicate that these mechanisms may vary considerably among species. Ultimately, whether catch or CPUE can be used to index abundance will depend on a clear-

[2] Bacheler, N., and L. Coggins. 2012. Unpubl. data. Beaufort Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA, Beaufort, NC 28516.

[3] Goldman, S. 2012. Personal commun. Marine Resources Monitoring, Assessment, and Prediction Program, South Carolina Department of Natural Resources, Charleston, SC 29422.

er understanding of the mechanisms that cause gear saturation.

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Effects of pot fishing on the physical condition of snow crab (*Chionoecetes opilio*) and southern Tanner crab (*Chionoecetes bairdi*)

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Abstract—The effects of commercial fishing with crab pots on the physical condition of the snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*) were investigated in the Bering Sea and in Russian waters of the Sea of Okhotsk. In crabs that were subjected to pot hauling, the presence of gas embolism and the deformation of gill lamellae were found in histopathological investigations. Crab vitality, which was characterized subjectively through observation of behavioral responses, depended on not only the number of pot hauls but also the time between hauls. Immediately after repeated pot hauls at short time intervals (≤3 days), we observed a rapid decline in vitality of crabs. When hauling intervals were increased to >3 days, the condition of crabs did not significantly change. After repeated pot hauls, concentration of the respiratory pigment hemocyanin ([Hc]) was often lower in the hemolymph of crabs than in the hemolymph of freshly caught animals. Our research indicated that changes in [Hc] in crabs after repeated pot hauls were caused by the effects of decompression and not by starvation of crabs in pots or exposure of crabs to air. We suggest that the decrease in [Hc] in hemolymph of snow and southern Tanner crabs was a response to the adverse effects of decompression and air-bubble disease. The decrease in [Hc] in affected crabs may be a result of mechanisms that regulate internal pressure in damaged gills to optimize respiratory circulation.

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Commercial fishing for crabs in most countries is currently carried out with the use of baited crab pots. With this method of catching crabs, it is possible to reduce the effects of fishing on crab populations by sorting the catch, because females, sublegal-size males, and crabs with missing legs are returned to the sea alive. However, some of the discards die from body injuries and physiological stress associated with temperature changes and exposure to air. In surviving crabs, physiological imbalance and altered behavior can affect their ecological functions, such as predator avoidance, feeding, migration, and reproduction. Crabs are discarded in high proportions in relation to landings, and comprehensive information on the fate of the discards and their survival rate is required to make management decisions in a fishery. Prediction of discard survivability under a wide range of fishing conditions requires fundamental knowledge of the effects of catch-related stressors on crab physical condition.

To date, many studies have examined the effects of stressors, such as trauma from the hauling of pots, air exposure, and adverse temperatures, that are associated with capture, handling, and discarding of crabs (van Tamelen, 2005; Tallack, 2007; Stoner, 2009; Darnell et al., 2010). Decompression due to fast transport through the water column is one of the stressors that affect crabs during fishing with pots. Decompression results in air-bubble disease, the formation of gas bubbles in the blood and body fluids of crabs (Ryazanova, 2009). Gas bubbles form in the hemal system, organs, and tissues of crabs, causing hemal stasis, disruption and displacement of tissues. Although decompression is a constant and unavoidable adverse factor associated with pot fishing, relatively little attention has been paid to its effects on crab condition. Traditionally, decompression is not considered to be a substantial source of discard mortality (Stoner, 2012). However, gas-bubble disease caused by decompression could have prolonged deleterious effects on crab condition because air bubbles may persist in the body of a crab for a long time (Johnson, 1976).

The subjects of this study were snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*). Both species are heavily fished along the continental shelf of the Bering Sea and the Sea of Okhtosk of the Russian Federation. Snow and southern Tanner crabs are shallow-water species. They generally live from subtidal areas to a depth of ~450 m, although snow crab has also been found in much deeper water (Jadamec et al., 1999; Mihailov et al., 2003; Slizkin, 2010). In Russian waters, the snow crab inhabits environments with a temperature range from –1.8°C to 7°C. The southern Tanner crab is usually found in temperatures of 2–3°C, avoiding bottom areas where a cold intermediate layer persists for a long time (Slizkin et al., 2001; Slizkin, 2010). In the area of overlap where the 2 species occur together, the abundance ratio of these crabs is strongly variable.

Unlike many other crustaceans, snow and southern Tanner crabs undergo a terminal molt before they become mature adults (Conan and Comeau, 1986; Comeau and Conan, 1992; Sainte-Marie et al., 1995). After their terminal molt, adult males of these species are available to pot fishing for only 0.5–3.0 years because their carapaces become progressively fouled and ultimately deteriorate. Also after their terminal molt, crabs can no longer grow and regenerate lost limbs. Adult males that lack a limb are returned to the sea after a catch is sorted and because discarded crabs may be re-injured, the number of individuals that lack limbs increases in heavily fished populations. High-density aggregations of female snow and southern Tanner crabs are found to occur in different areas from those where males are caught. Therefore, female snow and southern Tanner crabs are rarely encountered in catches. The percentage of undersize males (carapace width <100 mm) usually does not exceed 5–10% of the total catch because of selectivity characteristics of trap gear. Therefore, damaged and "dirty" male snow and southern Tanner crabs may often compose a significant portion of the discards that occur in pot fishing. Although these crabs do not have market value, they may be involved in reproduction. It is probable that these crabs may be recaptured multiple times throughout their life and, therefore, may be exposed potentially to cumulative effects of fishing procedures.

This study was designed to investigate the effects of decompression and other stressors associated with pot hauling on the physical condition of adult male snow and southern Tanner crabs that had completed a terminal molt between 1.5 years and a few months previously. We conducted our experiments in the field. Crabs taken from the commercial catch were placed in commercial pots. The pots were then sunk and hauled back to the surface at different frequencies. With this approach, multiple factors in addition to decompression can affect the survivability of animals. These other factors include significant changes in environmental conditions during hauls, interaction of crabs with the gear, and exposure to the air. Therefore, to specifically assess the effects of decompression on crab physical condition, histopathological changes in organs and tissues of crab caused by air-bubble disease were studied. Our approach was derived from the histological evidence of Johnson (1976), who found that the gills, heart, and antennal gland were the organs of blue crab (*Callinectes sapidus*) most severely affected by exposure to water supersaturated with air to simulate decompression effects. Bubbles persisted in the gills longer (more than a month) than in other organs of blue crab. We assumed that gill dysfunction caused by decompression would greatly affect the survivability of crabs after their release. Because of the logistical constraints of direct experimental research on gill function in the field, we investigated physiological parameters that could be used to estimate gill function disorders.

We selected hemocyanin (Hc) as the indicator for the assessment of the effects of pot fishing on the physical condition of the snow crab and southern Tanner crab. Hc is a copper-containing respiratory pigment that increases solubility of oxygen (O_2) by a factor of 2–4 and accounts for from 70% to more than 90% of the total protein concentration in the hemolymph of crustaceans (Truchot, 1992). Structural and functional variability of crustacean Hc has been shown to be of great importance for the adaptive condition of these organisms in response to metabolic changes and environmental stimuli (Bridges, 2001; Giomi and Beltramini, 2007). Therefore, we hypothesized that physiological alterations caused by impairment of respiratory function of the gills of snow and southern Tanner crabs could be correlated with changes in the structure and concentration of Hc. Plasma ion concentrations—for sodium ion (Na^+), potassium ion (K^+), chlorine ion (Cl^-), calcium ion (Ca^{2+}), and magnesium ion (Mg^{2+})—also were measured to examine ionoregulatory changes at the gills of affected crabs. During our experiments, factors other than decompression had an effect on crabs, including starvation and air exposure. Therefore, we also conducted experiments to assess the separate effects of starvation and air exposure on Hc.

We had the following objectives: 1) the investigation of histopathological deterioration of organs and tissues in snow and southern Tanner crabs subjected to pot hauling; 2) an analysis of the relationship between hauling intensity (the number and frequency of hauling events) and animal stress responses, including changes in behavior and biochemical parameters of hemolymph; and 3) an analysis of the utility of biochemical assays of Hc for prediction of survivability of snow and southern Tanner crabs subjected to hauling and discarding processes.

Materials and methods

Study areas

Snow crab and southern Tanner crab were collected during 5 fishing voyages in 2006, 2008, and 2010 in

both the Bering Sea (Fig. 1) and the Sea of Okhtosk (Fig. 2). In the areas where we conducted our investigations, surface temperatures typically vary from 4°C to 12°C in summer and from −1.5°C to 3°C in winter. Surface salinities generally vary between 32.2‰ and 33.0‰. In area I of the Bering Sea (Fig. 1) and in area I of the Sea of Okhtosk (Fig. 2), the 2 species occur together. At crab habitat depths of 50–350 m, water temperatures range from −0.5 to 5.5°C throughout the year and salinity varies from 33.2‰ to 34‰.

Crab capture and handling

Collection of crabs and experimental work were carried out on a crab boat. The fishing gears that we used in this study were square pots of the "American" type and conical pots of the "Japanese" type (Moiseev, 2003). Both types of pots were covered with nylon-twine webbing (mesh size: 60–70 mm). Crabs caught with commercial pots were maintained in large tanks supplied with natural seawater. Water temperatures during the holding period ranged from 4°C to 10°C. These tanks were covered with tarpaulins to reduce stress effects of light on the crabs. All individuals were tagged with a piece of rubberized fabric marked with a unique number; a tag was attached to a crab claw with a plastic clip-strip. Because of the tendency for autotomy of limbs in southern Tanner and snow crabs, all manipulations were carried out quickly with the greatest possible care. During holding periods, no crabs died or showed signs of stress in the form of impaired reflexes (Moiseev et al., 2012).

For this study, experiments were limited to crabs that met the following criteria: 1) morphometrically mature males had completed a terminal molt between 1.5 years and a few months previously, according to criteria in Comeau and Conan (1992) and Sainte-Maria et al. (1995); 2) carapace width for snow crab was 110–150 mm, and carapace width for southern Tanner crab was 120–155 mm; 3) crabs were without apparent physical injuries or limb loss; and 4) crabs were in perfect condition as revealed with a vitality index (VI).

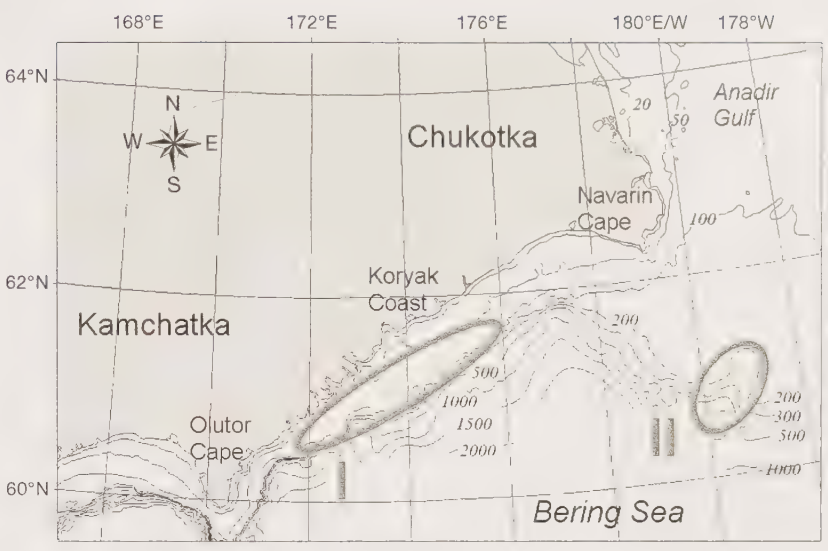

Figure 1

Map of areas where experiments and sample collection were conducted in the western Bering Sea in 2006–10 for our study of effects of commercial fishing with crab pots on the physical condition of the snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*). Area I was near the Koryak Coast, and area II was the shelf and slope near Cape Navarin.

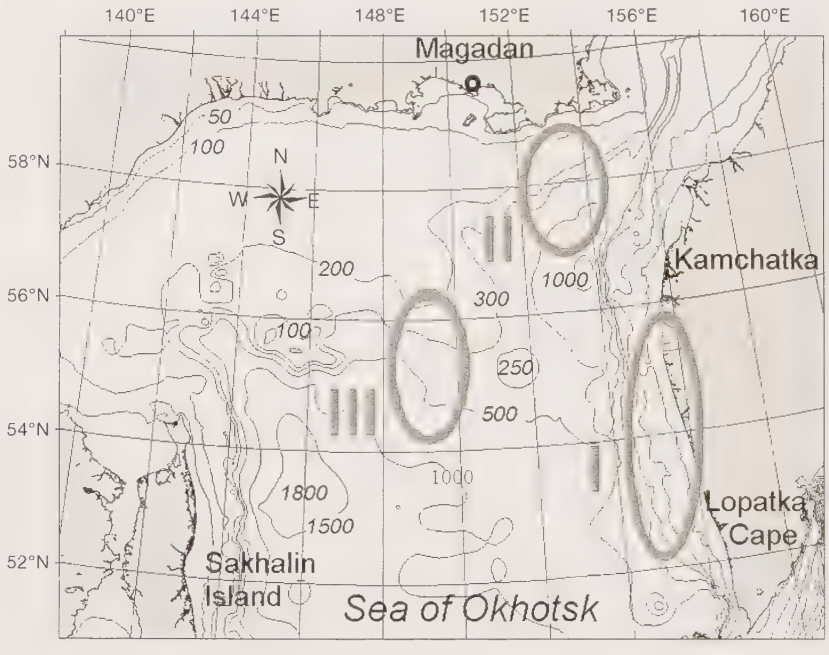

Figure 2

Map of areas where experiments and sample collections were conducted in the Sea of Okhtosk in 2006–10 for our study of effects of commercial fishing with crab pots on the physical condition of the snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*). Area I was on part of the Western Kamchatka shelf, area II was on the shelf and continental slope on the beam of the Babushkin Gulf, and area III was in the central area of the northern Sea of Okhtosk.

Table 1

Dates, locations, depths, hauling conditions, and sampling of hemolymph in experiments on snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*) conducted during 2006–10 in the Bering Sea and in Russian waters of the Sea of Okhotsk to study the effects of commercial fishing with crab pots on the physical condition of these species.

Experiment number	Year	Date	Areas[1]	Depth (m)	T°C at depth	Hauling intervals min/max (day)	Duration of experiment (day)	No. of pot lifts	Crab species	No. of crabs in a pot		Hemolymph sampling (No. of crabs)	
										Totals	Dead	Control[2]	Experiment[3]
1	2006	18 May–12 June	Bering Sea area I	75–125	0.0–1.0	4/16	25	3	*C. opilio*	20	4	15/5	15/5
									C. bairdi	21	5	18/5	16/5
2	2006	27 May–12 June	Bering Sea area I	85	0.3–1.0	16	16	1	*C. opilio*	5	–	–	3
									C. bairdi	6	–	–	3
3	2006	23 June 7 July	Sea of Okhtosk area II	190	1.5–2.0	7	14	2	*C. opilio*	32	5	16/5	12
4	2006	23 June–7 July	Sea of Okhtosk area II	190	1.5–2.0	14	14	1	*C. opilio*	28	2	11	7
5	2008	2–11 Oct	Sea of Okhtosk area I	85–110	2.0–3.5	2/3	9	4	*C. opilio*	13[4]	1	6	6
6	2008	6–11 Oct	Sea of Okhtosk area I	85–110	2.0–3.5	2/3	5	2	*C. bairdi*	23[4]	–	5	6
7	2008	17 Oct–3 Nov	Sea of Okhtosk area II	275–350	1.5–2.0	1/4	17	9	*C. opilio*	31	9	12	22
8	2008	7 July–2 Aug	Bering Sea area I	80–100	1.2–2.0	25	25	1	*C. bairdi*	10	–	5	9
9	2008	19 Sept–12 Nov	Sea of Okhtosk area I	185	1.5–2.0	55	55	1	*C. opilio*	7	–	9	5
									C. bairdi	8	–	5	5
10	2010	28 Sept–2 Oct	Sea of Okhtosk area III	230–340	1.6–2.5	2	4	2	*C. opilio*	10	8	5/5[5]	–
		29 Sept–5 Oct				2	6	3	*C. opilio*	11	9	5/5[5]	1
		30 Sept–6 Oct				2	6	3	*C. opilio*	15	11	5/5[5]	3

[1] See Figs. 1 and 2.

[2] Numerator=the total number of crabs that were sampled for hemolymph; denominator=the number of crabs that were used in an experiment after control sampling of hemolymph.

[3] Numerator=the number of crabs that were sampled for hemolymph only once; denominator=the number of crabs that were repeatedly sampled for hemolymph (3 or 4 times).

[4] Some crabs escaped during an experiment: in experiment 5, 6 crabs; in experiment 6, 14 crabs.

[5] Numerator=the number of crabs that were sampled for hemolymph immediately after capture; denominator=the number of crabs that were sampled for hemolymph after exposure to air.

Field experiments

Effects of repeated pot hauling on crabs Snow crab and southern Tanner crab were placed in commercial crab pots. In experiments 1, 3, 5, 6, and 7 we used a single pot of the American-type (Table 1). Before deployment of the pots, the entrance tunnels of pots were sewn shut with webbing to prevent individuals in our experiment from escaping and other crabs and big animals (e.g., mammals, big fishes) from entering the pots from the environment. During our experiments, crabs were not fed. The pots were sunk and hauled back to the surface at different frequencies. During the preparation of pots for the next deployment, crabs were maintained in tanks with running seawater for periods from 10–15 min to 1–2 h. Experiments that involved repeated pot hauls were divided into 2 sets, depending on the hauling intervals: 1) short time intervals (≤3 days)—experiments 5, 6, and 7—or 2) long time intervals (>3 days)—experiments 1 and 3 (Table 1). Both control animals and those animals that were collected for experiments had been lifted to the water surface during capture. Hereafter, the number of pot lifts we specify includes only the lifts made during the experiment, unless otherwise stated.

Upon retrieval of pots, mortality was noted and the condition of each crab was monitored with the vitality index. Criteria for the index incorporated reflexes, spontaneous movements of appendages, and righting behavior (i.e., an animal's ability to turn from a ventrum-up position to normal orientation):

0 = no signs of life: no movement of legs or mouthparts;
1 = weak, slow movement of legs or maxillipeds, attempts at righting behavior, third pair of maxillipeds droop open and retract to cover small mouth parts when touched by pencil, chelae grab any object slightly and not for long (up to 30 s);
2 = moderate movement of legs, righting time of 10–60 s in water bath, fast movement of maxillipeds, chelae grab any object strongly and for long periods (up to 1.5 min);
3 = fast, active movement of legs, righting time of 5–40 s in water bath or on the sorting table, fast movement of maxillipeds, chelae grab any object strongly and for long periods (≥2 min), threat display.

Effects of long-term starvation in pots on crabs In experiments 2, 4, 8, and 9, to investigate the effects of starvation, pots containing snow crab or southern Tanner crab were soaked (i.e., pots were deployed in the sea) for extended periods of time (14–55 days) (Table 1). Experiment 2 was combined with experiment 1. In the course of experiment 1, after the second lift of the pot, we added 5 freshly caught snow crab and 6 southern Tanner crab. The pot was lifted to the surface 16 days later; ocean conditions caused this long hauling interval (Table 1). At the end of all these experiments, the amount of meat in crab limbs was assessed by visual comparison of the slices of merus after boiling versus schematic representation of slices of merus in crab with varying degrees of meat content (Borisov et al., 2003).

Effects of long-term exposure to air on crabs Experiment 10 was designed to investigate the effects of long-term exposure to air on snow crab. It was replicated 3 times (Table 1). Snow crabs were kept out of the water on the deck from 6 to 8 h after capture. Animals were covered with a tarpaulin, which periodically was irrigated with seawater to protect the crabs from desiccation. Surface water temperatures were 6–7°C, and air temperatures were 7–8°C. Thereafter, 10–15 individuals were placed in conical pots of the Japanese type, and these pots were deployed in the sea. In each repetition of the experiment we used a single pot which was hauled 2–3 times at intervals of 2 days.

Sampling of hemolymph

Sampling of hemolymph in crabs from commercial catches was completed within 30 min after a pot had appeared at the water surface, the length of time referred to hereafter as *immediately after capture*. Samples of hemolymph taken from crabs immediately after capture served as control samples. Most crabs from which control hemolymph samples were taken were discarded, but, in experiments 1 and 3, some crabs were used in the experiments after they had control samples of blood taken from them. In experiment 9, control samples of hemolymph were taken from crabs immediately after capture, as well as from the crabs exposed to air for 6–8 h before deployment of a pot. In most cases, crabs used for control samples of blood and crabs for our experiments were collected from the same commercial catches. In some cases, because of ocean conditions, crabs used for control samples of blood were collected from other commercial catches, but they always were collected from catches in the same fishing area at a distance ≤0.9 km from the experimental pots and always during the periods of our experiments. Upon retrieval of pots, during our experiments that involved repeated pot hauls, hemolymph was sampled from some of the crabs used in experiments; these individuals were discarded after blood was sampled. Only in experiments 1 and 3, crabs from which hemolymph samples were taken remain in the pot. In experiment 1, in some individuals, blood was sampled repeatedly (3 or 4 times).

Hemolymph (3–5 mL) was quickly sampled from individual crabs with a syringe through the arthrodial membrane at the base of the fourth or fifth pair of legs. All manipulations were carried out quickly and carefully (the duration of crab exposure to air was not more than 10 min). The hemolymph samples were frozen and stored below –30° while on board. For transport to the biochemistry laboratory, they were kept on ice. Before analysis, the samples of hemolymph were thawed at 4°C and centrifuged for 15 min at 12,000 rpm, and the supernatant was collected. In snow and southern Tan-

Table 2

Dates, locations, depths, hauling conditions, and sampling for histology during repeated pot hauls in experiments conducted in 2010 to study the effects of commercial pot fishing on the physical condition of snow and southern Tanner crabs (*Chionoecetes opilio* and *C. bairdi*) in the Bering Sea and in Russian waters of the Sea of Okhotsk.

Experiment number	Year	Date	Areas[1]	Depth (m)	°C at depth	Hauling intervals min/max (day)	Duration of experiment (day)	No. of pot lifts	Crab species	Total number of crabs sampled for histology: Immediately after capture	Total number of crabs sampled for histology: During repeated pot hauls
1	2010	4–11 Sept	Bering Sea area II	170–260	2.5–3.5	2/3	7	3	*C. opilio*	3	5
									C. bairdi	3	5
2	2010	28 Oct–2 Nov	Sea of Okhtosk area III	230–340	1.6–2.5	2/3	5	2	*C. opilio*	3	5

[1] See Figures 1 and 2.

ner crabs, unlike in many other crabs, flocculent precipitate is formed during blood coagulation and can be removed easily by centrifugation.

Histological examinations

To examine the histopathological condition of organs and tissues in crabs that were subjected to repeated pot hauls, tissue samples were taken from both crab species during repeated pot hauls at time intervals of 2–3 days (Table 2). In each experiment, samples were taken from 5 individuals used in that experiment and from 3 freshly caught individuals that served as control animals. Fragments of internal organs were fixed for 24–40 h with Davidson's fluid (Bell and Lightner, 1988) prepared in seawater. After fixation, the samples were transferred to 70% alcohol, in which they were kept in the laboratory. This material was further treated with standard histological techniques: samples were dehydrated in an alcohol series of increasing concentrations, clarified with chloroform, and embedded in paraffin. Sections were stained with Meyer's hematoxylin-eosin (Johnson, 1980) and slides were examined under an Olympus Al-2 light microscope (Olympus Corp., Tokyo, Japan[1]).

Ionic composition of hemolymph

Colorimetric tests (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) were used to measure concentrations of Ca^{2+} ($[Ca^{2+}]$), Mg^{2+} ($[Mg^{2+}]$), and Cl^- ($[Cl^-]$) in hemolymph. Concentration of K^+ ($[K^+]$) and Na^+ ($[Na^+]$) were determined by flame photometry (FLAPHO 4, Carl Zeiss, Jena, Germany).

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Hemocyanin concentration

The [Hc] in the hemolymph of individual crabs was estimated with an established spectrophotometric method (Nickerson and Van Holde, 1971; deFur et al., 1990). An aliquot of serum was diluted by 50 mmol L^{-1} Tris HCl, containing 10 mmol L^{-1} EDTA, pH 8.9 (1:39). The absorbance of the resultant mixture was measured at 338 nm. The [Hc] was calculated with the extinction coefficient given by Nickerson and van Holde (1971), by assuming an average molecular weight of 75 kDa for subunits of hemocyanins (Hc's) from snow crab and southern Tanner crab.

Electrophoresis

To estimate the molecular weight of the different subunits of the Hc's from snow crab and southern Tanner crab, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) was carried out on whole hemolymph by using the discontinuous buffer system of Laemmli (1970) on slab gels. The stacking gel was made of 4.0% and 2.7% acrylamide and bis-acrylamide, respectively, with a pH of 6.8, and the resolving gel was made of 10.0% and 2.7%, respectively, with a pH of 8.8. The samples (10 µg) were run at 4°C and 20 mA with a tris-glycine buffer (pH 8.3). The gels were stained with Coomassie Brilliant Blue (Bio-Rad Laboratories, Hercules, CA).

Copper content in the hepatopancreas

In experiments 6 and 7, which involved repeated pot hauls at short time intervals (≤3 days), samples of hepatopancreas were dissected, after blood was sampled (Table 1). Samples of hepatopancreas also were dissected from southern Tanner crab after starvation in

the pot for 55 days in experiment 9 (Table 1). Samples of hepatopancreas were frozen in plastic bags, stored, and transported in the same manner as that used for the samples of hemolymph. The hepatopancreas copper content of individual crabs was determined by atomic absorption spectrophotometry (Shimadzu AA-6800, Shimadzu Corp., Kyoto, Japan) with the method of Kirichenko et al. (2005). In experiments 6 and 7, copper content was determined in samples of hepatopancreas from only those individual snow crab and southern Tanner crab in which [Hc] was twice as low as the mean [Hc] in the crabs immediately after capture.

Results

Dependence of crab vitality on haul frequency

Our experiments, in which we examined the physiological capacity of snow crab and southern Tanner crab to survive the variable environment associated with haul and return to the sea revealed that the condition of an animal was strongly dependent on the time interval between pot lifts. During repeated pot hauls at short time intervals (≤3 days) in experiments 5, 6, and 7, we observed a rapid reduction in reflex responses and locomotor activity in crabs. After 2–3 lifts in pots, the vitality of crabs was close to the minimum (VI=0–1). In experiment 7, the total number of pot lifts was 9. After the first 2 lifts, VI=1–2; after the third to sixth lifts, VI=0–1; and after the last 2 lifts, VI approached 0. During repeated pot hauls at long time intervals (>3 days) in experiments 1 and 3, the condition of the crabs did not significantly change or was only moderately suppressed (VI=2–3). At time intervals of more than a week between pot hauls (experiments 2, 4, 8, and 9), the condition of most crabs was not significantly different from the condition of freshly caught animals.

Changes in hemocyanin concentration depending on hauling interval

Long intervals (>3 days) In experiment 1, 3 pot lifts were made: the first lift on the fifth day, the second lift on the ninth day, and the third lift only on the 25th day because of ocean conditions (Table 1). We observed a significant decrease in [Hc] in the hemolymph of snow crab and southern Tanner crab during this experiment: both in crabs in which blood was sampled only once (Fig. 3, A and B) and in crabs in which blood was sampled more than once (Fig. 4, A and B). The main decrease in [Hc] occurred during the time that elapsed between the capture of crabs and the second pot lift. During the next 16 days and up to the 25th day of the experiment, further decreases in [Hc] were observed but were not significant (Fig. 3, A and B).

In experiment 3, mean [Hc] in the hemolymph of snow crab used in our experiments decreased by 51%, after the first pot lift on the seventh day, compared with the mean [Hc] found in control samples collected immediately after capture, and the difference was significant (Student's t-test, $P<0.01$). After the second pot lift on the 14th day, large variations in [Hc] in the hemolymph of snow crab were observed, and the difference between experimental and control animals, therefore, was not significant (data not shown).

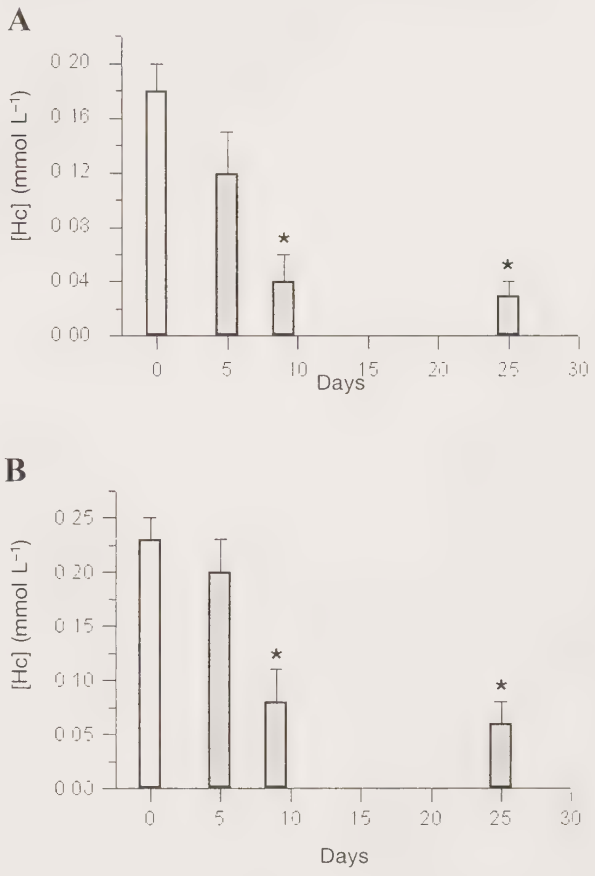

Figure 3

Changes in mean hemocyanin concentration [Hc] in (A) southern Tanner crab (*Chionoecetes bairdi*) and (B) snow crab (C. opilio) sampled for hemolymph only once during repeated pot hauls at long time intervals (>3 days) in experiments conducted on 18 May–12 June 2006 in area I of the Bering Sea to study the effects of commercial pot fishing on the physical condition of these species (see Fig. 1 for location of area I). Open bars indicate mean [Hc] observed in crabs sampled immediately after capture (18 individuals for southern Tanner crab and 15 for snow crab), and shaded bars indicate mean [Hc] observed in crabs sampled after repeated pot hauls (for both species of crabs, 4–7 individuals were sampled after each pot haul). Asterisks (*) indicate values significantly different by Student's t-test ($P<0.01$), compared with mean [Hc] observed in crabs sampled immediately after capture. Error bars indicate ±1 standard error of the mean.

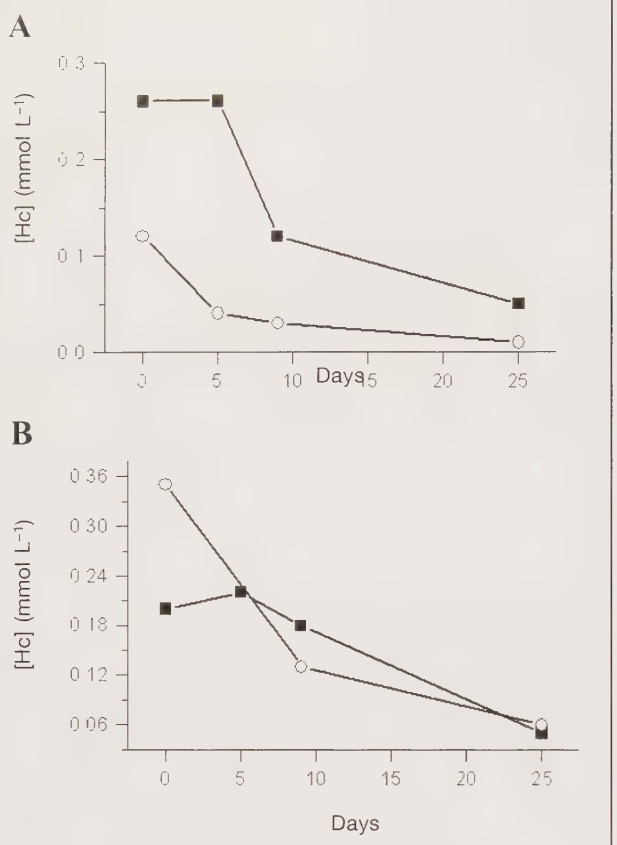

Figure 4

Changes in hemocyanin concentration [Hc] in (A) southern Tanner crab (*Chionoecetes bairdi*) and (B) snow crab (*C. opilio*) repeatedly sampled for hemolymph during multiple pot hauls at long time intervals (>3 days) in experiments conducted on 18 May–12 June 2006 in area I of the Bering Sea to study the effects of commercial pot fishing on the physical condition of these species (see Fig. 1 for location of area I).

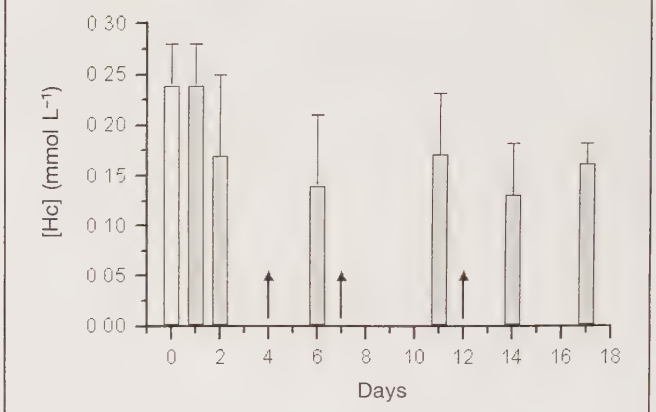

Figure 5

Changes in mean hemocyanin concentration [Hc] in snow crab (*Chionoecetes opilio*) during repeated pot hauls at short time intervals (≤3 days) in experiments conducted on 17 October–3 November 2008 in area II of the Sea of Okhtosk to study the effects of commercial pot fishing on the physical condition of these species (see Fig. 2 for location of area II). In experiment 7, ocean conditions extended time intervals to 1–4 days rather than the planned 1–2 days. Open bar indicate mean [Hc] observed in crabs sampled immediately after capture (number of individuals was 12), and shaded bars indicate mean [Hc] observed in crabs sampled after repeated pot hauls (the number of individuals sampled after each pot haul was 3–5). Error bars indicate ±1 standard error of the mean. Arrows indicate third, fifth, and seventh pot lifts when sampling of blood was not carried out.

Short time intervals (≤3 days) In experiment 7, the planned time intervals between lifts were 1–2 days. However, because of ocean conditions, they ranged from 1 to 4 days. The total number of pot lifts was 9, and hemolymph was not sampled after the third, fifth, and seventh lifts (Table 1). Large variations in [Hc] in the hemolymph of snow crab were observed after the second pot lift until the end of the experiment (Fig. 5). In one-third of experimental animals, [Hc] was markedly lower, by 50% or more, than mean [Hc] observed in control crabs, although, in the remaining animals used in the experiment, [Hc] did not differ significantly from Hc levels in control samples.

Experiments 5 and 6 were similar to but not as prolonged as experiment 7. In both of these experiments, large variations in [Hc] in snow and southern Tanner crabs were observed. In experiment 5, in approximately one-half of individual snow crab, [Hc] was markedly lower, by 80% or more, than mean [Hc] in control crabs. In experiment 6, in approximately one-half of individual southern Tanner crab, [Hc] was markedly lower, by 70% or more, than mean [Hc] in control crabs. In both of these experiments, in one-half of experimental animals, [Hc] did not differ significantly from Hc levels in control samples (data not shown).

Effects of long-term starvation on crabs in pots

In experiment 2 and 4, the duration of pot soaking was 16 and 14 days, respectively. At the end of these experiments, mean [Hc] in hemolymph of snow crab and southern Tanner crab did not significantly differ from mean [Hc] in hemolymph of control crabs immediately after capture (data not shown).

In experiment 8, the duration of pot soaking was 25 days. At the end of this experiment, mean [Hc] in hemolymph of southern Tanner crab was slightly lower than [Hc] in control crab immediately after capture, but the mean difference between experimental and control animals was not significant (Student's t-test, $P>0.05$) (Fig. 6A).

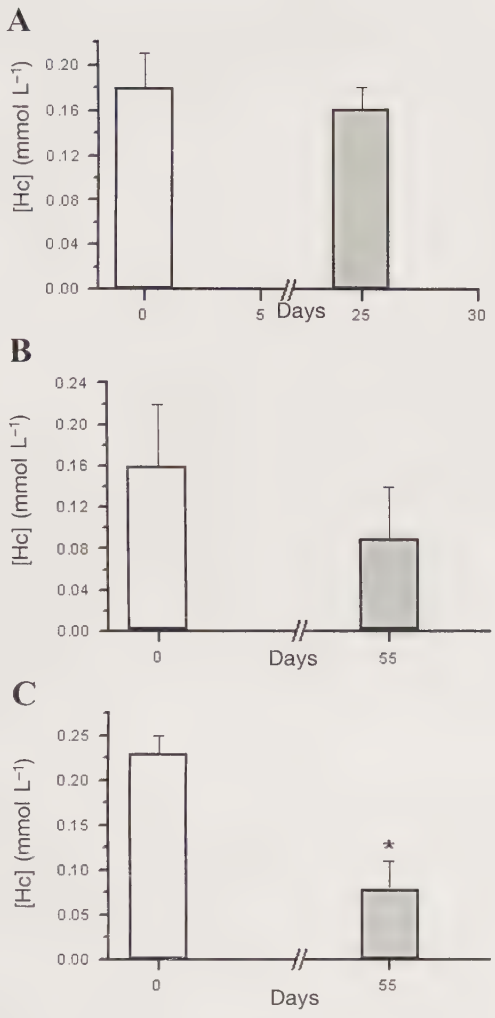

Figure 6

Changes in mean hemocyanin concentration [Hc] in snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*) after starvation in crab pots. Open bars indicate mean [Hc] observed in crabs sampled immediately after capture (9 individuals for snow crab and 5 for southern Tanner crab for each experiment), and shaded bars indicate mean [Hc] observed in crabs sampled after starvation (5 individuals for snow crab and 9 and 5 for southern Tanner crab for experiments with pot soaking time of 25 days and 55 days, respectively): (**A**) southern Tanner crab, pot soaking time=25 days, Bering Sea (area I), 7 July–2 August 2008; (**B**) southern Tanner crab, pot soaking time=55 days, 19 September–12 November 2008, Sea of Okhtosk (area I); and (**C**) snow crab, pot soaking time=55 days, 19 September–12 November 2008, Sea of Okhtosk (area I). An asterisk (*) indicates values were significantly different by Student's *t*-test ($P<0.001$), compared with mean [Hc] observed in crabs immediately after capture. Error bars indicate ±1 standard error of the mean.

In experiment 9, pots soaked for 55 days. At the end of this experiment, mean [Hc] in southern Tanner crab decreased by 44% compared with mean [Hc] in control crab immediately after capture (Fig. 6B), but the difference between the experimental and control animals was not significant (Student's *t*-test, $P>0.05$). In snow crab, mean [Hc] decreased by 67% compared with mean [Hc] in control crab (Fig. 6C), and the difference was significant (Student's *t*-test, $P<0.001$).

During experiment 4, 2 crab died. At the end of the remaining experiments, all experimental animals were alive and their vitality was high (VI=2–3). At the end of all experiments, the weight of experimental crabs and the amount of meat in crab limbs did not change significantly.

Effects of long-term exposure to air on snow crab

In experiment 10, after long-term exposure to air, the vitality of snow crab was low (VI=0–1) and mean [Hc] in these animals was comparable with the mean [Hc] in control crabs immediately after capture. After long-term exposure to air, most of the crab that were subjected to the pot-hauling process were dead after the first pot-hauling event. Some individuals were able to survive 2–3 pot hauls (Table 1). Samples of hemolymph could be obtained from crab only after the first pot haul. [Hc] in hemolymph of those animals was comparable with the mean [Hc] in control crab immediately after capture.

Histological investigations

In our experiments both control and experimental animals were hauled from the water in the pot at least once and, therefore, gas embolism was observed in hearts and gills of both control and experimental animals during gross examination of dissected crabs (Fig. 7). Histopathological investigations found intact and deformed gill lamellae in both experimental and control individuals of snow crab and southern Tanner crab (Fig. 8, A and B; Fig. 9, A–C). Hemolymph spaces of deformed gill lamellae were greatly expanded, sometimes in the form of vesicular swellings, or, conversely, the opposite walls of lamellae were stuck together (Fig. 9A). Often the connections of pillar cells in dilated parts of lamellae were torn (Fig. 9B). Marginal canals had irregular shapes or were swollen and filled with a large number of hemocytes. Opposite walls of lamellae under the marginal canals, as a rule, were stuck together (Fig. 9C). Gas bubbles and aggregations of hemocytes were found in the myocardia of individuals of both experimental and control snow crab and southern Tanner crab. Similar changes often were observed in antennal glands, in skeletal muscles, and in connective tissues of the stomachs and guts of crabs. Infiltrations of hemocytes and different-size, melanized inclusions often were found in the gill lamellae of both control and experimental crabs (Fig. 9D). We were unable to find or show dependence

Figure 7

Photographic image showing air-gas bubbles (↑) found in the median shaft of the gill of a southern Tanner crab (*Chionoecetes bairdi*) during gross examination of dissected crabs as part of our study of the effects of pot fishing on the physical condition of *Chionoecetes* crabs in the Bering Sea and Russian waters of the Sea of Okhotsk.

of the degree of damage in gills on the number of lifts (Table 2).

Copper content in the hepatopancreas

A significant difference in hepatopancreas copper levels between control and experimental individuals (Student's t-test, $P<0.05$) was observed in experiment 9, where long-term starvation of southern Tanner crab in a crab pot was investigated (Table 3). There were no significant differences in hepatopancreas copper levels between control and experimental snow crab and southern Tanner crab in experiments 6 and 7, which involved frequent lifts of crab pots.

Ionic composition of hemolymph

The ionic composition of hemolymph from snow and southern Tanner crabs under different experimental conditions was investigated (Table 4). In experiment 1, a significant decrease in $[K^+]$ was observed in hemolymph from snow crab (Student's t-test, $P<0.01$) and southern Tanner crab (Student's t-test, $P<0.05$) after 3 pot hauls. In experiment 9, a significant decline in $[K^+]$ was observed in hemolymph from snow crab after starvation in a pot for 55 days (Student's t-test, $P<0.05$).

Subunit composition of hemocyanins

In the Hcs from snow crab and southern Tanner crab, 4 and 3 differently migrating polypeptide chains, respectively, were found in the SDS/PAGE experiment. In our investigations, the patterns of bands obtained by SDS/PAGE in both crab species did not change under different experimental conditions (data not shown).

Discussion

Effects of pot fishing on crab vitality

In most studies on the effects of pot fishing on crabs, the main focus was mortality of animals. Experiments in other studies that involved repeated pot hauls showed that mortality of deepwater beni-zuwai crab (*Chionoecetes japonicus*) and triangle Tanner crab (*Chionoecetes angulatus*) depended on molt stage, and mortality of new-shell crabs was greater than mortality of old-shell animals (Koblikov, 2004; Vasilyev and Kli-

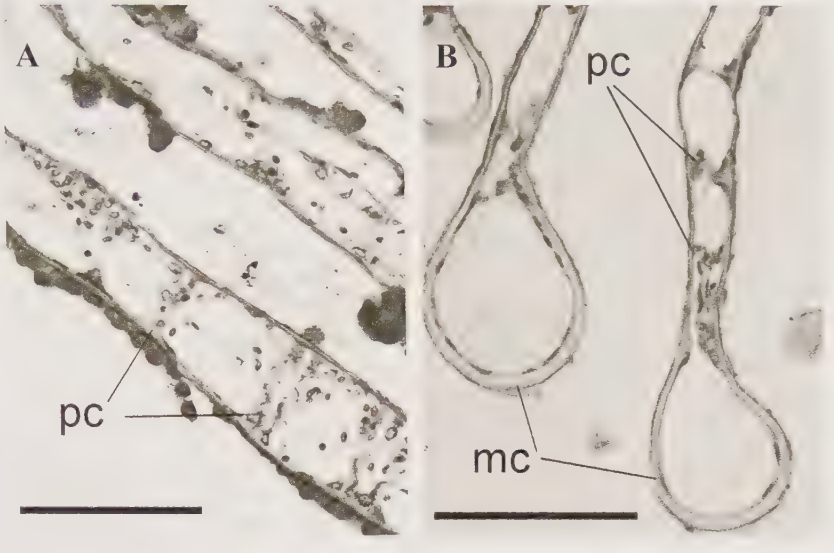

Figure 8

Light micrographs of normal gills in snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*) from our study of the effects of pot fishing on the physical condition of these species: (A) distal part of lamellae in a southern Tanner crab and (B) apical part of lamellae in a snow crab. Hematoxylin and eosin staining was used. Abbreviations: pc=pillar cells; mc=marginal canal. Scale bar=100 μm.

nushkin, 2011). Tallack (2007) investigated mortality of red deepsea crab (*Chaceon quinquedens*) in different hauling conditions (i.e., crabs hauled every 24 h, after intervals of 4 or 8 days) and found that crab hauled to the surface every 24 h showed significantly greater mortality than crab retrieved after 4 or 8 days.

In our study, experiments on snow and southern Tanner crabs, like all experiments described in the previous paragraph, were conducted as actual field operations with minimal control of external variables, including air exposure, changes in temperature, light, and physical injuries. In our experiments, mortality of crabs was induced by blood sampling. For example, in experiment 1, mortality was 20% for snow crab and 24% for southern Tanner crab, and all the dead crabs that could be identified from their remains were crabs from which samples of blood were taken 1 or 2 times. This mortality most likely resulted from predation of amphipods (e.g., *Orchomenella affinis*) that were attracted to the wounds caused by sampling. However, in experiment 3, mortality rates did not differ significantly between crabs from which samples of blood were taken (17%) and crab from which no blood samples were taken (15%).

Another substantial source of crab mortality in our experiments was trauma associated with gear encounters. Externally visible injuries to the carapace were found often in dead animals that had encountered gear. In all dead crabs, soft tissues were quickly eaten away by predatory amphipods. It can be assumed that, within the size range of crabs kept in the pots in our experiments (see the *Materials and methods* section), aggressive interaction between crabs and cannibalism were not significant factors in the mortality of animals. Cannibalism in snow crab occurs only between individuals of significantly different sizes (Dutil et al., 1997; Lovrich and Sainte-Marie, 1997). Our research on the attractiveness of dead crabs as bait showed that they did not attract individuals of their own species (senior author, unpubl. data).

Therefore, mortality of crabs in our experiments was not a reliable indicator of the physiological disorders in crabs. Reversible physiological disorders did not result in death of the animals, and mortality of crabs often was caused by trauma from gear. During the course of our experiments in the field, the vitality of each crab was monitored with a VI. Vitality, or general health status of crabs, can be characterized subjectively by observing or testing behavioral or whole-animal responses (reflex actions). The severity of physiological disorders in crabs is related directly to changes in vitality of crabs (Stoner, 2009, 2012).

The haul and return of pots to the seabed caused shifts in the homeostasis of crabs, leading to changes in health status of the animals. In turn, the health status of the animals influenced their physical responses to stress. We used adult male snow crab and southern Tanner crab that had completed a terminal molt between 1.5 years and a few months previously. Therefore, the vulnerability of animals in our research to external factors was less influenced by intrinsic variables, such as processes of growth, maturation, or molt stage, than it would have been if we had used immature or egg-producing female crabs.

A temporary shift of physiological homeostasis in crabs caused by the process of pot hauling may be corrected by the natural regulatory capacity of an organism or by adaptive physiological responses. The investigations on the mortality of snow crab in crab pots carried out by Ivanov and Karpinski (2003) revealed that crab weakened by frequent pot hauls could restore natural physiological conditions (i.e., locomotor activity and righting behavior) if the time intervals between successive pot lifts were increased. In their experiments, it was found that after 2 pot lifts at time intervals of 2 days, the surviving crabs were greatly weakened. However, the condition of animals improved significantly af-

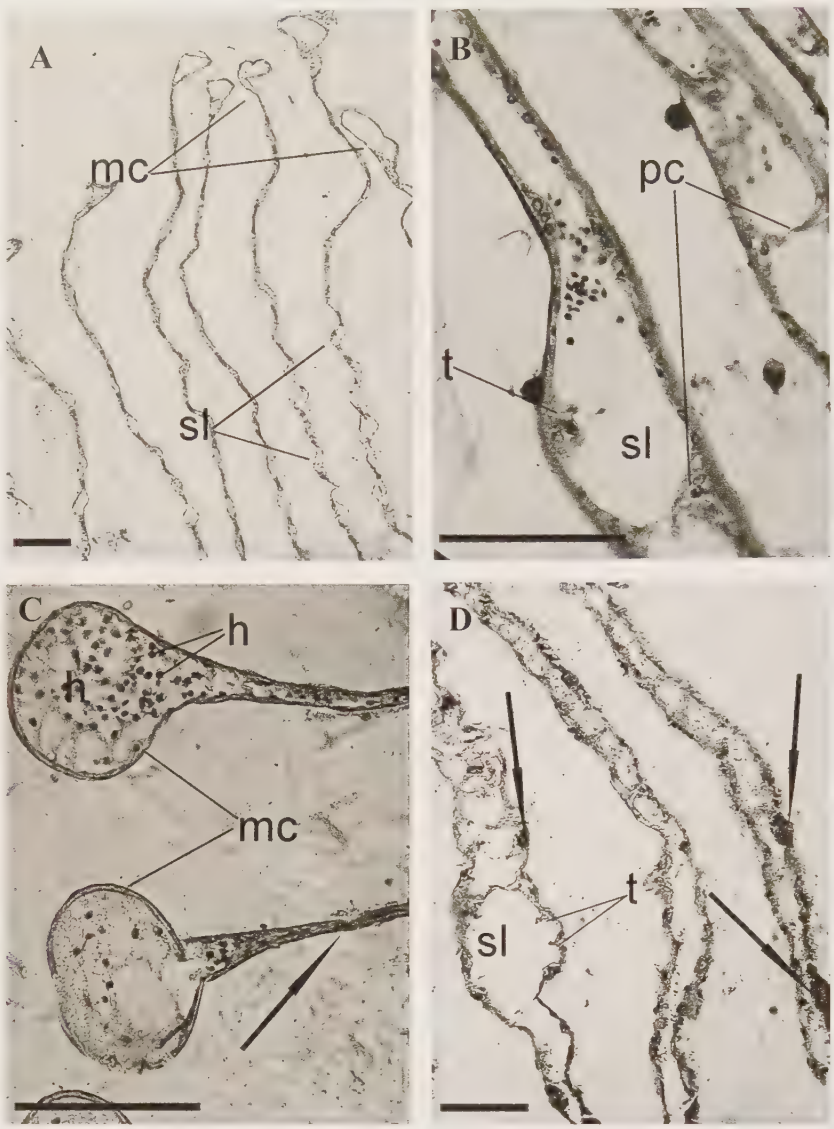

Figure 9

Light micrographs of damages to gills in snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*) from our study of the effects of pot fishing on the physical condition of these species: (A) deformation of lamellae; note the irregular shape of the marginal channels and vesicular swellings; (B) torn connections of pillar cells in the dilated parts of the lamellae; (C) swollen marginal canals filled with hemocytes and collapsed lamellae walls (marked by arrow); and (D) melanized inclusions in lamellae (arrows); note the deformation of lamellae and torn connections of pillar cells. Hematoxylin and eosin stains were used. Abbreviations: mc=marginal canal; sl=swelling of lamellae; pc=normal pillar cells; t=torn pillar cells; and h=hemocytes. Scale bar=100 µm.

ter the pot had soaked for 4 days. After an additional 8 days of pot soaking, crabs recovered completely: all animals were alive and their condition did not significantly differ from the condition of freshly caught animals.

In our experiments, comparisons of the different hauling conditions also revealed that the vitality of animals was strongly dependent not only on the number of but also on the length of time between pot lifts. During repeated pot hauls at short intervals (≤3 days), we observed a rapid decline in vitality of crabs. When we increased hauling intervals to >3 days, the condition of crabs did not change significantly or was suppressed only moderately.

Effects of pot fishing on hemocyanin concentration

In our experiments, [Hc] was selected as an objective measurement for the assessment of the effects of pot fishing on the physical condition of snow crab and southern Tanner crab. In addition to serving as a practical and simple indicator, it can be easily related to respiratory function, which can be impaired because of air exposure and gill disorders that result from fishing and handling operations.

The [Hc] in crustacean hemolymph is determined by the balance between anabolism and catabolism of the protein. The turnover rate of crustacean Hc is thought to be slow in usual steady-state conditions. Half-times for clearance of ^{125}I-labeled Hc have been reported to be 25.5 and 36 days in American lobster (*Homarus americanus*) and Caribbean spiny lobster (*Panulirus argus*) (Senkbeil and Wriston, 1981). A more rapid synthesis of Hc can be induced by various environmental factors, such as changes of ambient salinity (Gilles, 1977; Péqueux et al., 1979) and by moderate chronic hypoxia (Hagerman and Baden, 1988; deFur et al., 1990; Spicer and Baden, 2001).

A decrease in [Hc] in hemolymph of crustaceans may be caused by starvation. It has long been noted that [Hc] is related to the nutritional state of crustaceans under natural conditions (Dall, 1974; and references therein), leading some researches to suggest that, in times of plenty, Hc serves not only as an O_2 carrier but also as a storage protein. Terwilliger (1998) noted, "This may occur, although storing ex-

Table 3

Copper concentration, [Cu], measured in micrograms (g wet weight^{-1}), in samples of the hepatopancreas of snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*) under different experimental conditions. [Cu] values are means (±1 standard error [SE]).

	C. bairdi[1]		*C. opilio*[2]	
Experimental conditions	[Cu]	No. of samples	[Cu]	No. of samples
Immediately after capture	5.5 (1.3)	7	0.92 (0.07)	7
Starvation in a crab pot within a period of 55 days	37.3 (18.5)*	5	–	–
Repeated pot hauls at short time intervals (≤3 days)	17.0 (12.1)	6	2.6 (1.8)	6

[1] 19 September–12 November 2008, 6–11 October 2008, Sea of Okhtosk (area I).
[2] 17 October–3 November 2008, Sea of Okhtosk (area II).
* Significantly different from that of individuals sampled immediately after capture ($P<0.05$), Student's t-test.

cess nutrients as circulating oligomeric proteins seems a bit expensive" (p. 1088). Nonetheless, studies have shown that concentrations of total protein and Hc in hemolymph steadily decrease in starved animals under laboratory conditions (Hagerman, 1983; and references therein). However, it is probable that some of these effects were a result not of reduction in the absolute amount of protein but of dilution of the protein. Dall (1974) showed that blood protein concentration in the longlegged spiny lobster (*Panulirus longipes*) decreased with starvation within 4 weeks, but this decrease was actually due to the increase in blood volume as solid tissues were metabolized. Djangmah (1970) observed that blood protein decreased from 7.9 g L^{-1} to 2.55 g L^{-1} and that there was a progressive accumulation of copper in the hepatopancreas of the common shrimp (*Crangon crangon*) after 23 days of starvation. He suggested that the stored copper came from catabolism of Hc, which is, therefore, rendered usable as an energy source in a starving animal. Laboratory studies showed that the changes of [Hc] elicited by food availability occurred gradually over periods of many days (Djangmah, 1970; Hagerman, 1983). However, Spicer and Strömberg (2002) demonstrated that starvation had a significant effect on [Hc] in northern krill (*Meganyctiphanes norvegica*) over the course of a few hours (≤10) under laboratory conditions. They used laboratory studies to interpret changes in krill [Hc] that they observed during the diel vertical migration of northern krill. Spicer and Strömberg (2002) attributed such dramatic decreases in [Hc] in starved individuals to the high metabolic rates of northern krill.

During our experiments, crabs were not fed because it was not possible to control the amount of food consumed by individuals. Any food placed in crab pots is quickly eaten by predatory amphipods, which also are thought to attack weak or injured crabs (Ivanov and Karpinski, 2003). Because the duration of our experiments was up to several weeks, there was a need to determine to what extent the fall in crab [Hc] was related to the forced starvation of animals during the experiments. In our experiments on the effects of starvation on crabs, at pot soaking durations of 14, 16, and 25 days, [Hc] in snow crab and southern Tanner crab did not change significantly compared with [Hc] in crabs immediately after capture (Fig. 6A). A significant drop in mean [Hc] between crabs before and crabs after soaking was observed only in experiment 9, in which crabs soaked in a pot for 55 days (Fig. 6, B and C). We, therefore, assume that starvation had no significant effect on the changes in the [Hc] in crabs in all experiments that involved repeated pot hauls because, in these experiments, [Hc] significantly changed over the course of a few days.

A rapid decrease in [Hc] in crustaceans may be associated with the processes of anaerobic metabolism, which is one of the major physiological responses to O_2 deprivation. Increases of lactate and bicarbonate-carbonic acid during anaerobic metabolism result in increased hydrogen ion concentration and a drop in pH in crustacean hemolymph (Barrento et al., 2009; and references therein). Crustaceans have compensation mechanisms that act in response to acidosis through mobilization of buffer bases. However, during severe and prolonged hypoxia, the ability of an organism to compensate may be exhausted. When no further metabolic compensation occurs, the pH level will drop below the tolerated range and, as a result, the rates of enzymatic reactions, ionic and osmoregulatory controls, and cell membrane structure will be affected. Poor health, morbidity, and mortality have been reported to be correlated with low pH values in crustaceans (Whiteley and Taylor, 1992; Ridgway et al., 2006).

In laboratory experiments, Norway lobster (*Nephrops norvegicus*) exposed to water with low concentration of dissolved O_2 (<20% O_2-saturation) showed a sudden and rapid decrease in [Hc] (Baden et al., 1990). The length of time before the decline began depended on the O_2 concentration. When lobster were exposed to 10% O_2-saturation, an immediate reduction of their mean [Hc]

Table 4

Ionic composition of hemolymph from snow and southern Tanner crabs under different experimental conditions. Ionic concentration values are means (±1 standard error [SE]). *n*=no. crabs sampled.

Species	Experimental conditions	Ion concentration (mmol L^{-1})									
		Na^+	*n*	K^+	*n*	Ca^{2+}	*n*	Mg^{2+}	*n*	Cl^-	*n*
	Seawater from Bering Sea	449	–	9.5	–	9.8	–	51.1	–	523	–
	Seawater from Sea of Okhtosk	453	–	9.6	–	10.5	–	51.6	–	528	–
C. opilio	Repeated pot hauls at long time intervals (>3 days)[1]										
	Immediately after capture	465 (13)	4	12.6 (0.4)	7	8.3 (0.3)	16	36.0 (0.6)	10	482 (4)	10
	After 3rd lift	443 (28)	3	9.2 (0.7)**	6	8.0 (0.4)	8	34.0 (1.5)	8	469 (17)	8
	Long-term starvation in the crab pot (55 days)[2]										
	Immediately after capture	399 (15)	7	10.8 (0.5)	7	8.2 (0.4)	7	35.7 (1.5)	7	482.8 (19)	7
	After starvation	374 (33)	5	8.7 (0.9)*	5	7.2 (0.6)	5	32.6 (3.8)	5	463 (48)	5
C. bairdi	Repeated pot hauls at long time intervals (>3 days)[1]										
	Immediately after capture	472 (5)	4	13.0 (0.8)	7	7.7 (0.3)	18	33.8 (0.7)	10	477 (5)	10
	After 3rd lift	388 (78)	3	8.2 (2.1)*	6	7.5 (0.5)	7	30.8 (2.2)	7	444 (28)	7
	Long-term starvation in the crab pot (25 days)[3]										
	Immediately after capture	406 (25)	3	11.3 (0.6)	4	8.2 (0.3)	5	27.0 (1.1)	5	413 (38)	5
	After starvation	386 (28)	3	8.9 (0.1)	3	8.3 (0.4)	9	29.4 (0.8)	9	400 (25)	9
	Long-term starvation in the crab pot (55 days)[2]										
	Immediately after capture	367 (19)	6	10.0 (0.8)	6	7.7 (0.4)	6	31.1 (2.2)	6	458 (28)	6
	After starvation	370 (23)	5	9.5 (1.1)	5	7.7 (0.3)	5	32.4 (2.7)	5	447 (34)	5

[1] 18 May–12 June 2006, Bering Sea (area I).
[2] 19 September–12 November 2008, Sea of Okhtosk (area I).
[3] 7 July–2 August 2008, Bering Sea (area I).
* Significantly different from that of individuals sampled immediately after capture ($P<0.05$), Student's *t*-test.
** Significantly different from that of individuals sampled immediately after capture ($P<0.01$), Student's *t*-test.

by 65% of the initial value occurred within 4 days. Animals in water with 12% and 15% O_2-saturation first increased their [Hc] for 15 and 36 days, respectively. After those time periods, a rapid decrease in [Hc] occurred at a rate similar to the one recorded for lobster in water with 10% O_2-saturation. Mortality of animals during hypoxic experiments ranged from 40% to 100%. Glycogen depletion in muscles and in the hepatopancreas of lobster also was seen after O_2 deficiency, reflecting a shift to anaerobic metabolism (Baden et al., 1994). However, loss of functional hemocyanin in the hemolymph of lobsters during these hypoxic experiments (as indicated by a reduction in oxyhemocyanin concentration measured with a spectrophotometer) did not correlate with loss of blood copper. Baden et al. (1994) suggested that, under conditions of hypoxic experiments, the functional integrity of Hc molecules is damaged, leading to changes of the O_2-binding properties of Hc. Their assumption seems reasonable because severe metabolic acidosis induces an increase in protein degradation because of activation of proteolysis and nonenzymatic hydrolysis of proteins.

In our experiments, respiratory acidosis, internal hypoxia, and anaerobic metabolism in crabs may have been caused by impaired gas exchange in damaged gills or exposure to air during handling on the deck. However, in our experiments, declines in [Hc] were not related to decreases in animal vitality. The highest decrease in [Hc] for snow and southern Tanner crabs was observed in the experiments with repeated pot hauls at long time intervals (>3 days), during which the vitality of animals was high. In animals considerably weakened during repeated pot hauls at short time intervals (≤3 days) or during prolonged air exposure, [Hc] was reduced to a lesser extent or not changed (Figs. 3, 4, and 5).

The rapid decline in [Hc] observed in our experiments was, therefore, unlikely to have been due to degradation of the protein as a result of respiratory hypoxia and severe metabolic acidosis. Additionally, SDS/PAGE showed no changes in the subunit composition of Hc's from snow crab and southern Tanner crab in our experiments. In individuals of both species of crabs, different proportions of the Hc subunits were sometimes observed, but these differences were not correlated with

experimental conditions. Therefore, the structure of the Hc's remained unchanged in our experiments.

Changes in [Hc] in our experiments, therefore, were likely caused by physical and functional impairments that resulted from pot hauling. Changes in vitality of animals and [Hc] depended not only on the number of pot lifts but also on the time intervals between them. Decreases in [Hc] were related to high vitality of crabs. These observations indicate that the physiological imbalances caused by repeated pot hauls can be corrected if the time interval until the next haul is sufficiently long. It is also likely that the stress response involves changes in [Hc].

Copper content in the hepatopancreas

It has been postulated that copper metabolism in decapod crustaceans associated with degradation of Hc (during starvation or before molting) includes the processes of copper translocation to the hepatopancreas and storage in the form of complexes with glutathione and copper-binding proteins (Djangmah, 1970; Brouwer et al., 2002). Brouwer et al. (2002) showed that metabolism of the copper-binding protein metallothionein in the hepatopancreas of blue crab was related to molt stage. Metallothionein isoform 3 was present in premolt and softshell crab and was absent in hepatopancreas of intermolt crab. Emergence of this protein appears to coincide with a decrease in Hc synthesis and increase in Hc degradation.

In experiment 9, after starvation within a period of 55 days, a significant increase in mean copper concentration in the hepatopancreas of southern Tanner crab was found. We also observed a significant decrease in mean [Hc] in these crabs, compared with Hc levels in control animals immediately after capture. However, in experiments 6 and 7, which involved repeated pot hauls at short time intervals, there were no significant changes in hepatopancreas copper levels in snow and southern Tanner crabs that had reduced [Hc] (Table 3). Therefore, much of the copper that was released during Hc degradation caused by pot hauling did not accumulate in the hepatopancreas and was probably excreted from the body. This result may be explained by low levels of copper-binding proteins in the hepatopancreas of adult males of snow and southern Tanner crabs because they do not have periods of active synthesis and degradation of Hc associated with molt stage.

Ionic composition of hemolymph

Plasma ion (Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}) concentrations were measured to examine ionoregulatory changes at the gills of affected crabs. The snow and southern Tanner crabs are typical of other stenohaline marine crustaceans in that their hemolymph is isosmotic with their environmental seawater but the ionic composition of their hemolymph can differ considerably from the composition of seawater around them (Prosser, 1973). In the hemolymph of snow crab and southern Tanner crab, $[K^+]$ is considerably higher and $[Mg^{2+}]$ is considerably lower than concentrations in the surrounding seawater.

Biochemical analysis of stress responses in crustaceans have shown that various stressors can alter ion concentrations in hemolymph and, therefore, that ions may be used as indicators of physiological stress (Stoner, 2012; and references therein). However, the exact mechanisms and physiological causes of ionic changes are usually difficult to explain. Several studies have reported increased $[Ca^{2+}]$ and concentration of bicarbonate ion (HCO_3^-) in crustacean hemolymph during aerial exposure, which may indicate the mobilization of calcium carbonate ($CaCO_3$) from internal sources, such as calcified exoskeleton to compensate for low pH (Taylor and Whiteley, 1989; Lorenzon et al., 2007). $[Mg^{2+}]$ can be elevated in hemolymph of open-wounded crabs because of entry of magnesium-rich seawater (Uhlmann et al., 2009). Changes in $[K^+]$, $[Na^+]$, and $[Cl^-]$ in hemolymph of crustaceans are highly variable with stress (Stoner, 2012; and references therein).

In experiment 1, which involved repeated pot hauls at long time intervals, and in experiment 9, which investigated long-term starvation in pots, $[K^+]$ in hemolymph of snow crab and southern Tanner crab were significantly lower compared with $[K^+]$ in freshly caught crabs (Table 4). It should be noted that, in experiments 1 and 9, mean [Hc] in hemolymph of snow crab and southern Tanner crab were significantly lower compared with mean [Hc] in control crab immediately after capture.

An essential role is played by K^+ in the maintenance of the difference in electrical potential across the plasma membrane of a cell, typically referred to as the "membrane potential." That physicochemical regulatory function enables normal nerve impulse transmission, normal contraction of muscle fibers, and normal heart function. Interestingly, changes in the ionic composition of the hemolymph of crabs after repeated pot hauls were the same as changes in the ionic composition during long-term starvation, when metabolic rate and physical activity probably were reduced. However, the meaning of these changes needs to be studied.

Effects of decompression on crab condition

Changes in ambient pressure during lifts of crabs in pots to the water surface are an unavoidable adverse factor that affects the crabs. Studies of the blood circulation of brachyuran crabs, including snow and southern Tanner crabs, indicate that fluctuations of ambient pressure have the greatest effect on the blood flowing in the gills (Taylor, 1990). The phyllobranchiate gills of brachyuran crabs consist of 2 alternating rows of closely spaced, flattened lamellae that extend forward and backward from a median shaft. Each lamella consists of single layer of epithelial cells lining the cuticle. Pillar cells, which are located in the cellular layers in oppo-

site walls of lamellae are connected to each other halfway in the hemolymph space. As common gill epithelial cells, pillar cells perform respiratory or salt transporting function depending on their localization within the gills and also are thought to play a role in stabilizing the lamellae against ambient and internal hydrostatic pressure (Compere et al., 1989; Johnson, 1980). Large fluctuations of ambient pressure can cause damage of the lamellae because the fragile pillar cells are not well adapted to withstand pressure forces. In our experiments, we observed disruption in the connections of pillar cells and collapse of the lamellae in gills of snow crab and southern Tanner crab that were subjected to repeated pot hauls (Fig 9).

The phyllobranchiate gills of brachyuran crabs are supplied with low pressure venous hemolymph. The afferent to efferent drop in pressure and mean internal pressure are generally only a few centimeters of water (Blatchford, 1971). During normal forward ventilation of gills by the scaphognathites, the lamellae tend to be inflated by a small positive transmural pressure, which is the difference between the above-ambient pressure in the lamellar hemocoel and subambient mean pressure in the branchial chamber in crab. Under these conditions, the resistance to hemolymph flow through the circulatory channels in the gills of crabs is low (McMahon and Burnett, 1990). However, any damage to gill structure could lead to a significant increase in gill resistance and consequently to increases in both the drop in pressure through the gills and in mean internal pressure (Taylor, 1990). Increased pressure in the injured gills likely would cause further gill damage and even greater impairment of gill function.

The main factor that determines the internal pressure in the gills of crabs is thought to be the total volume of internal fluid. One of the mechanisms for adjustment of a total fluid volume is regulation of urine release (Taylor, 1990). Hc is the major extracellular protein of crab hemolymph; hence, it is [Hc] that determines colloid osmotic pressure of blood plasma. In intermolt marine crabs, the small excess of hydrostatic pressure of hemolymph over colloid osmotic pressure drives passive filtration, which forms primary urine (Mangum and Johansen, 1975). The decrease in [Hc] may elevate the driving force for urine formation and, consequently, may lead to a decrease in total volume of internal fluid in crabs. In our experiments, blood sampling from crabs became more difficult after repeated pot hauls because the exhaust velocity of hemolymph from the cut was reduced. At the same time, the clotting time of hemolymph in experimental crabs was not changed or was increased compared with the clotting time in control animals (data not shown). These data indirectly indicate a decrease in blood pressure in the leg sinus that may be caused by a decrease in total volume of hemolymph.

A decrease in total blood volume through a decrease in [Hc] could not only prevent further gill damage but also reduce cardiovascular workload. However, a decrease in [Hc] causes a decline in the O_2-carrying capacity of hemolymph that, in combination with impaired gas exchange in the damaged gills, could lead to a further decrease in supply of O_2 to organs and tissues. Studies of the role of crustacean Hc in O_2 transport have found complete oxygenation of Hc in postbranchial hemolymph but only partial deoxygenation of Hc in the tissues of normoxic, routinely active animals. The oxygenation level of Hc in the prebranchial hemolymph of resting normoxic animals (often called *venous reserve*) usually amounts to 50% or more of the O_2 capacity of the hemolymph. Reduced O_2 supply to the blood transport system or increased metabolic demand could result in depletion of the venous reserve. In normoxic conditions, the contribution of Hc to O_2 transport significantly increases during physical exercises (e.g., locomotor activity) of animals (Truchot, 1992; and references therein).

In our previous studies of [Hc] in the hemolymph of red king crab (*Paralithodes camtschaticus*) in the Barents Sea and in the Sea of Okhtosk, [Hc] during the molt cycle was closely correlated with the volume of the limbs filled with muscular tissue (Moiseeva and Moiseev, 2008; Moiseeva and Moiseev, 2011). Those results also indicate that the O_2 delivery system with Hc is required primarily to support high levels of locomotor activity of crabs. Therefore, reduction of [Hc] and a simultaneous decrease in locomotor activity of crabs could optimize blood flow in gills damaged by fisheries operations without a significant decrease in supply of O_2 to organs and tissues.

Decompression, which results from a rapid lift of a pot to the surface of the water, is not a natural environmental stressor for crabs. However, our experiments showed that crabs quite successfully adapted to the damage of organs and tissues caused by decompression because they took advantage of existing physiological mechanisms. The gills of crabs are damaged often under natural conditions. In our experiments, in September–October 2010 in the northern Sea of Okhtosk, gross examination of tissues and organs revealed areas of melanization and necrosis in the gills of 40% of 50 freshly caught snow crab that had no sign of shell disease. In some crab, parts of some of the gills were absent (data not shown). Histological examination of organs and tissues has revealed histopathological changes in gills in 95% of snow crab with signs of shell disease (Ryazanova, 2006).

Gills serve an important role in the immune response of crustaceans. Aggregates of hemocytes and bacteria appear in hemolymph in response to microbial pathogens and then become trapped in the narrow hemolymph spaces of gills, where they are melanized and persist for a long time. The formation of hemocyte aggregates in blue crab that were injected with bacteria led to increases in vascular resistance across the gills and subsequent significant increases in the drop of hydrostatic pressure across the gill circulation (Burnett et al., 2006). Therefore, impairment of respiratory circulation apparently occurs often during infectious and noninfectious gill disorders of crabs. Crabs would be expected to possess

mechanisms to cope with such impairments. The rapid degradation of Hc that we observed in our experiments may be one of these mechanisms. Increased degradation of blood protein, especially of Hc, has been observed in several euryhaline crab species during hyperosmotic stress (Gilles, 1977). In our experiments on snow and southern Tanner crabs, a similar phenomenon was observed in stenohaline crabs for the first time.

A single lift in a pot to the water surface apparently does not have significant effects on a crab. All of the crabs in our experiments survived at least 1 pot lift, the lift when they were captured. Therefore, experiments 8 and 9 on long-term starvation for 25 and 55 days, respectively, also can be considered for study of the delayed mortality of crabs after decompression. At the end of these experiments, all the animals were alive and had a high vitality. The negative effect of decompression significantly increases if the same crab is recaptured within a few days. However, the probability of such an event is very low. Crabs normally move actively in search of food; however, it is unlikely that discarded crabs will do so while they recover from their first capture.

On the basis of our experiments, a crab can survive (without serious harm) a recapture event that occurs more than a few days after its first capture. However, a decrease in [Hc] due to stress response would pose a challenge for snow and southern Tanner crabs. If its O_2 transport system fails to meet all of the demands for energy during locomotion, a crab would not be able to sustain its normal level of activity. The reduction in [Hc] in crabs after a single lift in a pot obviously would depend on the initial condition of the animals and external factors. As shown in our experiments, this reduction in [Hc] can be very significant.

Several studies have shown that the synthesis of Hc in crustaceans depends on the quality of food eaten (Hagerman, 1983). It is assumed that food, not seawater, is the most important source of copper in crustaceans (Baden, 1990; and references therein). Copper reserves in the hepatopancreas may be used to reconstitute Hc, but significant accumulation of copper was not found in our experiments in the hepatopancreas of snow and southern Tanner crabs during degradation of Hc due to decompression. Therefore, the recovery of [Hc] in affected crabs would depend greatly on the availability and quality composition of the fodder base in their habitat.

Conclusions

Our research has shown that pot fishing has a significant effect on the physical condition of snow and southern Tanner crabs. Gill damage caused by ambient pressure differences and gas-bubble disease could have long-term effects on the vitality of crabs returned to sea from commercial catches after sorting. Both species of crabs have effective mechanisms for physiological adaptation to the adverse effects of pot fishing. The decrease in [Hc] that we observed in affected crabs is a long-term adjustment of the internal fluid volume of crabs that reduces pressure in damaged gills and optimizes respiratory circulation. The vital activity of crabs returned to sea after sorting of catches would depend on their condition before capture and the challenges presented by the specific environmental conditions at the time of their release.

Biochemical assays of Hc have proved to be a useful tool for the investigation of the effects of pot fishing on the physical condition of snow crab and southern Tanner crab. The plasticity of Hc content constitutes an efficient mechanism for crustaceans to cope with metabolic or environmental challenges. In our experiments, the greatest changes in [Hc] that resulted from stress response to impairment of blood flow in gills occurred in the most viable crabs. Therefore, [Hc] can be a useful indicator of the health of crabs in conditions that can lead to gill dysfunction.

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Reproductive potential of Yellowfin Tuna (*Thunnus albacares*) in the western Indian Ocean

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Abstract—The reproductive biology of Yellowfin Tuna (*Thunnus albacares*) in the western Indian Ocean was investigated from samples collected in 2009 and 2010. In our study, 1012 female Yellowfin Tuna were sampled: 320 fish on board a purse seiner and 692 fish at a Seychelles cannery. We assessed the main biological parameters that describe reproductive potential: maturity, spawning seasonality, fish condition, and fecundity. The length at which 50% of the female Yellowfin Tuna population matures (L_{50}) was estimated at 75 cm in fork length (FL) when the maturity threshold was established at the cortical alveolar stage of oocyte development. To enable comparison with previous studies, L_{50} also was estimated with maturity set at the vitellogenic stage of oocyte development; this assessment resulted in a higher value of L_{50} at 102 cm FL. The main spawning season, during which asynchrony in reproductive timing among sizes was observed, was November–February and a second peak occurred in June. Smaller females (<100 cm FL) had shorter spawning periods (December to February) than those (November to February and June) of large individuals, and signs of skip-spawning periods were observed among small females. The Yellowfin Tuna followed a "capital-income" breeder strategy during ovarian development, by mobilizing accumulated energy while using incoming energy from feeding. The mean batch fecundity for females 79–147 cm FL was estimated at 3.1 million oocytes, and the mean relative batch fecundity was 74.4 oocytes per gram of gonad-free weight. Our results, obtained with techniques defined more precisely than techniques used in previous studies in this region, provide an improved understanding of the reproductive cycle of Yellowfin Tuna in the western Indian Ocean.

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Knowledge of reproductive traits is important for understanding population dynamics, including a population's resilience to fishing (Schaefer, 2001; Murua and Motos, 2006; Morgan et al., 2009). As alternatives to the traditional spawning stock biomass (SSB), reproductive potential indices have been proposed in which basic reproductive parameters are included as important factors that affect population productivity (Trippel, 1999; Morgan et al., 2009). These parameters include sex-ratio, the age and size of females, maturation ogive, fecundity, fish condition, and reproductive history. Inclusion of these biological parameters allows integration of fluctuations in a population's reproductive success into the assessment and management processes, in addition to estimation of spawning stock biomass (SSB) (Murua and Saborido-Rey, 2003; Murua et al., 2010). Hence, to improve the assessment and management of stocks, it is necessary to increase the quality and quantity of the basic reproductive data used to estimate these reproductive parameters (Korta, 2010).

Yellowfin Tuna (*Thunnus albacares*) is one of the major target species of the tuna fishery in the Indian Ocean. Total annual catch of Yellowfin Tuna in the Indian Ocean has increased significantly, since the early 1980s, with the advent of the purse-seine fishery. Average annual catch reached 473,896 metric tons (t) between 2003 and 2006 but decreased in 2007 and 2008 to around 320,000 t; in 2011, total annual catch was around 300,000 t (IOTC[1]).

The Yellowfin Tuna is a batch-spawner with asynchronous ovary organization (Schaefer, 2001) and indeterminate fecundity (Zudaire et al., 2013). This species can spawn with a frequency of around 1.5 days (McPherson, 1991; Schaefer, 2001) over a vast area of the tropical zone throughout the year (Itano[2]; Stéquert et al., 2001). Its spawning events, as with other tuna species, occur in rela-

[1] IOTC (Indian Ocean Tuna Commission). 2012. Report of the fifteenth session of the IOTC Scientific Committee. Victoria, Seychelles. IOTC Secretariat, P.O. Box 1011, Victoria, Seychelles.

tion to sea-surface temperature (>24°C), which seems to regulate spawning activity (Schaefer, 2001). In the Indian Ocean, spawning mainly occurs in the equatorial area (0–10°S) from December to March, and the main spawning grounds have been observed west of 75°E (IOTC[1]). However, other authors have reported variations in the spawning season of this species, describing periods between January and June (Zhu et al., 2008). Stéquert et al. (2001) related reproductive activity of Yellowfin Tuna with the north monsoon (main spawning period) from November to March and with the south monsoon (less reproductive activity) from June to August. Similarly, different estimates of maturity have been published for this species in several studies that used the same method to identify maturation stages. For example, the length at which 50% of females mature (L_{50}) was estimated at around 100 cm in fork length (FL) for Yellowfin Tuna in the Indian Ocean by the Indian Ocean Tuna Commission (IOTC[1]), at 108 cm FL in the western Pacific Ocean by McPherson (1991), at 92 cm FL in the eastern Pacific Ocean by Schaefer (1998), and at 104 cm FL for the equatorial western Pacific Ocean by Itano.[2]

In spite of the ecological importance of Yellowfin Tuna and the significant decrease of its catches in various regions during recent years, there have been few studies that have focused on the reproductive biology of the Indian Ocean Yellowfin Tuna. This study aims to contribute to the knowledge of various reproductive traits essential for examination of the reproductive potential and population dynamics of Yellowfin Tuna in the western Indian Ocean, with an estimation of the following qualities: 1) length at 50% maturity; 2) spawning season; 3) fecundity; and 4) fish condition during reproduction.

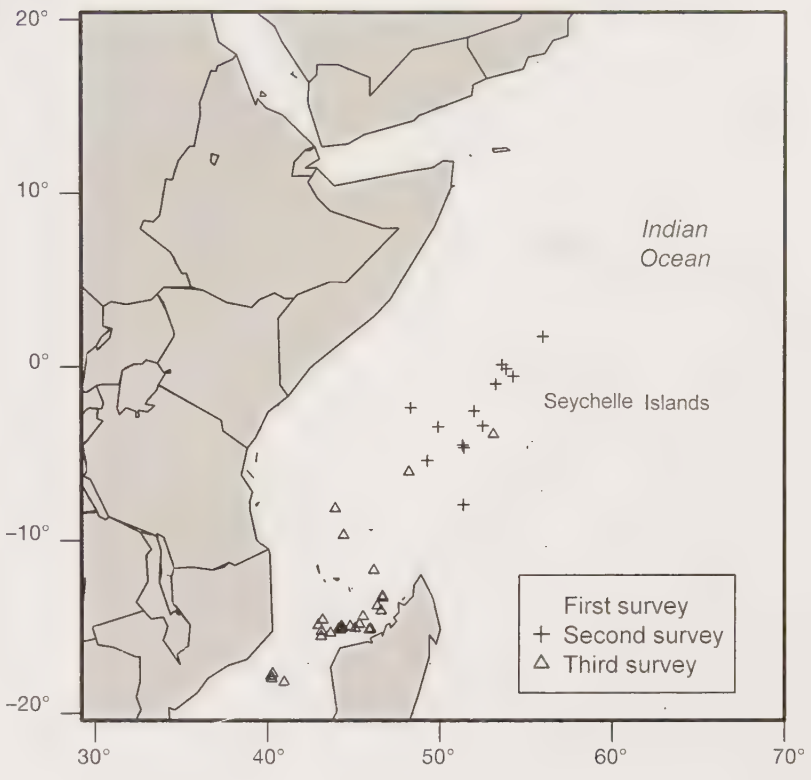

Figure 1

Map of locations where female Yellowfin Tuna (*Thunnus albacares*) were sampled in the first survey (22 January–23 March 2009), second survey (6 May–25 July 2009), and third survey (12 January–13 April 2010). The surveys were conducted aboard a commercial purse seiner in the western Indian Ocean to study the reproductive potential of Yellowfin Tuna in this region.

Materials and methods

Field sampling

Sampling was done by scientific observers during 3 surveys conducted on board a commercial purse seiner in the western Indian Ocean during 2009 and 2010 (Fig. 1). At sea, during these surveys, 320 female Yellowfin Tuna were sampled. In addition, 692 ovaries from female Yellowfin Tuna that were captured by the purse-seine fleet that operated in the western Indian Ocean during the same period were collected at the Seychelles cannery (Table 1). Yellowfin Tuna were identified at sea and in the cannery through the use of characters given in Collette and Nauen (1983). Fork length (in centimeters), total weight (in kilograms), sex, maturation stage and gonad weight (in grams) were recorded for each individual. Liver weight (in grams) was measured only in females that were sampled at sea. Their ovaries were removed and weighed to the nearest gram. A cross section of the gonad of 4–5 cm was cut between the middle and end part of the right or left lobe and preserved in 4% buffered formaldehyde immediately after collection until it could be processed in the laboratory.

In the laboratory, cross sections (~1 cm) from the preserved portions of 819 ovaries were embedded in resin, sectioned at 3–5 µm, and stained with hematoxilyn and eosin (Korta, 2010). The histological classification of Yellowfin Tuna ovaries followed the criteria

[2] Itano, D. G. 2000. The reproductive biology of yellowfin tuna (*Thunnus albacares*) in Hawaiian waters and the western tropical Pacific Ocean: project summary. Pelagic Fisheries Research Program (PRFP), Joint Inst. Mar. Atmospheric Research (JIMAR), Univ. Hawaii (UH), HI. JIMAR contribution 00–328, p. 69. JIMAR, MSB 312, 1000 Pope Road, Honolulu, HI 96822.

Table 1

Period of sampling, ranges of fork lengths of tuna sampled, number of females sampled in each survey, and number of females used in different analyses for our study of the reproductive potential of Yellowfin Tuna (*Thunnus albacares*) in the western Indian Ocean. Analyses included the histological analysis of ovaries (Hist.), analysis of fecundity (Fec.), and analysis of the condition indices of gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (*K*).

Survey	Sampling period	Length (cm)	Females sampled	Hist.	Fec.	GSI	HSI	*K*
1	22/01/2009–23/03/2009	37–158	114	110	6	106	105	106
2	5/06/2009–25/07/2009	30–161	95	95	5	95	77	95
3	03/04/2010–21/05/2010	31–157	114	114	–	95	94	95
Cannery	12/01/2010–13/04/2010	61–147	692	500	31	511	–	511

described in Zudaire et al. (2013). On the basis of terminology used in Brown-Petersen et al. (2011) and applied to Yellowfin Tuna (Zudaire et al., 2013), the ovaries were classified according to the most advanced oocyte stage present in the ovary: *immature phase* (including the primary growth stage [PG]), *developing phase* (including the cortical alveolar [CA], primary vitellogenesis [Vtg1], and secondary vitellogenesis [Vtg2] stages), *spawning-capable phase* (including the tertiary vitellogenesis [Vtg3], germinal vesicle migration [GVM], and hydration stages), and *regenerating phase*. The different stages of alpha-atresia were classified according to Zudaire et al. (2013). The ovaries collected at the cannery were not included in the analyses of atresia because they had been exposed to brine preservation used on board purse seiners, making it impossible to quantify alpha-atresia precisely. The ovaries collected at sea and at the cannery were analyzed for the identification of postovulatory follicles; however, no postovulatory follicles were found in these ovaries.

Length at 50% maturity

All females included in this analysis were staged histologically. L_{50} was calculated by fitting the proportion of mature females by 5-cm size classes to a logistic equation (Ashton, 1972; Saborido-Rey and Junquera, 1998):

$$P_{\text{mature}} = e^{\alpha+\beta L}/1+e^{\alpha+\beta L},$$

where P_{mature} = the predicted proportion of mature females;
L = the FL in centimeters; and
α and β are the coefficients of the logistic equation.

The L_{50} was estimated as the ratio of the coefficients ($-\alpha^*\beta^{-1}$). A nonlinear regression (the Marquardt method without restrictions; Marquardt, 1963) was used to fit the logistic equation to the data. The curve of L_{50} was estimated on the basis of the assumption that females with ovaries at the cortical alveolar stage onward were mature (Brown-Peterson et al., 2011). A second criterion was used to enable comparisons of our results with results reported in previous publications (Schaefer, 1998; Itano[2]; Zhu et al., 2008): females with cortical alveolar oocytes at the most advanced developmental stage were considered immature and females with advanced vitellogenic oocytes were considered mature.

Condition indices

Three condition indices were measured to estimate the condition of females: the gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (*K*). These 3 indices were defined in this manner:

$$GSI = (W_g/W) \times 10^2;$$
$$HSI=(W_l/W) \times 10^2;$$
$$K=(W/L^3) \times 10^2;$$

where Wg = gonad weight;
W_l = liver weight;
W = fish gonad-free weight in grams; and
L = FL in centimeters.

The seasonal variations in those indices and their effect on the reproductive cycle were analyzed by month. To identify possible physiological changes during the ontogeny of Yellowfin Tuna, the seasonal development of the 3 condition indices was analyzed in 2 size groups: fish <100 cm FL and fish >100 cm FL—the L_{50}'s adopted by the Indian Ocean Tuna Commission [IOTC[1]]). Tuna sampled at the cannery were not included in the HSI analysis because the livers of these specimens were not weighed.

Fecundity estimation

Batch fecundity (BF), the total number of oocytes released per batch, was estimated for 40 ovaries with the gravimetric method (Hunter et al., 1989) by counting the oocytes in the germinal vesicle migration or hydration stages. Homogeneity in oocyte density among

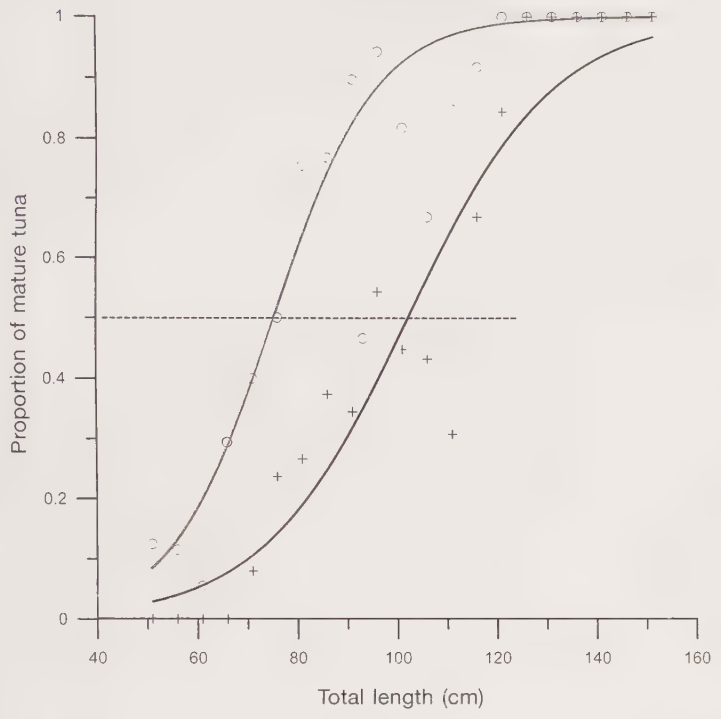

Figure 2

Proportion of mature female Yellowfin Tuna (*Thunnus albacares*) in the western Indian Ocean at 5-cm length intervals and fitted to a logistic regression curve in our study of the reproduction potential of this species in this region. Circles represent the proportions of females considered mature when their ovaries were at the cortical alveolar and later stages; the gray solid line indicates the logistic regression curve for these females. Crosses represent the proportions of females considered mature when their ovaries were at the vitellogenic and later stages; the dark solid line indicates the logistic regression curve for these females. The horizontal, dotted line indicates L_{50}, the length at which 50% of the female Yellowfin Tuna population were mature.

whole ovaries was assumed on the basis of previous studies on tuna (Stéquert and Ramcharrun, 1996). For BF analyses, 3 subsamples of 0.1 g (±0.01) were collected from each ovary. Each subsample was saturated with glycerin and oocytes were counted under a stereomicroscope (Schaefer, 1987, 1996, 1998). Batch fecundity was calculated as the weighted mean density of the 3 subsamples multiplied by the total weight of the ovary. A threshold of 10% for the coefficient of variance was applied for the 3 subsamples, and when this threshold was surpassed, more subsamples were counted to reach it. Relative batch fecundity (BFrel) was estimated by dividing the BF by the gonad-free weight of the fish. The relationships between the BF and BFrel with the FL, weight, and condition indices (GSI, HSI, and K) of females were determined. The seasonal trend in fecundity was analyzed through estimation of monthly mean BF and BFrel.

Statistical analyses

A nonparametric Kruskal-Wallis test (H-test) was applied to determine differences in the GSI, HSI, and K among months and between maturation stages. The relationships between the BF and BFrel and other biological parameters, such as length, weight, and condition indices (HSI and K), were analyzed through application of simple correlation and regressions. Analysis of variance (ANOVA) was applied to analyze the differences in the estimates of mean BF and mean BFrel by month during the spawning season.

Results

Length at 50% maturity

L_{50} was estimated to be 75 cm FL when females with ovaries at the CA stage and onward were considered mature. This estimate increased to 102 cm FL when the second criterion was applied (i.e., when the maturity threshold was defined as the presence of advanced vitellogenic oocytes, Fig. 2). In both cases, the proportion of mature females by length provided a good fit to the logistic model (coefficient of determination [r^2]=0.89 and r^2=0.91, respectively) (Table 2).

Reproductive cycle

The analysis of the female maturation process throughout the year showed that 30.1% of the individuals sampled were in the immature phase, 44.4% were in the developing phase, 20.3% were in the spawning-capable phase, and 5.12% were in the regenerating phase (Table 3). Overall, 69.8% of the sampled females were in the mature state, 92.6% of which were reproductively active.

The analysis of the maturation process was carried out for 2 size groups: individuals >100 cm FL and individuals <100 cm FL. The females >100 cm FL showed ovaries more developed and closer to spawning from November to February and in June than in other months (Fig. 3A). The period between November and January was especially important because more than 90% of the females sampled were in the spawning-capable phase. In contrast, during April and May, there was no spawning activity for this size group (fish >100 cm FL), with 40% and 30% of the ovaries in the regenerating phase in each month, respectively. The occurrence of immature phase ovaries increased to 50% and 38% in April and May.

Table 2

Summary of logit parameters of the female maturity (L_{50}=length at which 50% of the population is mature) curve for 2 criteria used in our study of Yellowfin Tuna (*Thunnus albacares*) reproduction in the western Indian Ocean. The symbols α and β represent the coefficients of the logistic equation, and r^2 is the coefficient of determination. In criterion 1, the L_{50} was calculated with the assumption that females with ovaries at the cortical alveolar stage onward were mature. The L_{50} in the criterion 2 was estimated with the assumption that females with cortical alveolar oocytes as the most advanced developmental stage were immature and those with advanced vitellogenic oocytes were mature.

	Criterion 1		Criterion 2	
Parameters	α	β	α	β
Estimate	−8.654	0.113	−6.965	0.068
Standard error	1.604	0.021	1.246	0.012
	Estimates		Estimates	
Number of females	423		423	
$L_{50}(-\alpha / \beta)$	74.7 cm		102.0 cm	
r^2	0.89		0.91	

The females <100 cm FL showed a considerable predominance of immature phase ovaries from April to November with levels of 50% or higher (Fig. 3B). These percentages of immature phase ovaries decreased below 30% during December, January, and February, and the percentage of ovaries in the spawning-capable phase increased to 4.4%, 5.3%, and 6.9%, respectively, for each month. It is noteworthy that ovaries in the regenerating phase appeared during the spawning season for females <100 cm FL, with values of 8.9% and 10.5% in December and January, but ovaries in this phase for larger Yellowfin Tuna (>100 cm FL) appeared in April and May.

Condition indices

The monthly variation in the GSI of the previously defined 2 size groups showed statistically significant differences between months for fish <100 cm FL (H_{669}=136.9, P<0.01) and fish >100 cm FL (H_{313}=206.4, P<0.01). Females >100 cm FL had a peak in mean GSI values that coincided with the highest proportion of females in the spawning-capable phase from November to January (GSI>2.0). Afterward, mean GSI values decreased sharply with a minimum value in April of 0.26 (standard deviation [SD] of 0.0). In June, the mean GSI increased again to 0.98 (SD 0.5) in a second spawning period but with lower reproductive activity compared with the main spawning period (Fig. 4A). In contrast, females <100 cm FL had almost constant mean GSI values except in December (0.44 [SD 0.2]), January (0.52 [SD 0.3]), and February (0.43 [SD 0.3]), when the GSI increased slightly during the main spawning period of large specimens of Yellowfin Tuna. There was no increase in the mean GSI in June for the smaller size group (Fig. 4B).

The monthly variation in HSI and K of both size groups showed statistically significant differences between months: HSI for fish <100 cm FL (H_{154}=29.1, P<0.01) and for fish >100 cm FL (H_{122}=26.3, P<0.01), and K for fish <100 cm FL (H_{669}=175.5, P<0.01) and for fish <100 cm FL (H_{313}=30.6, P<0.01). For females >100 cm FL, K values had a pattern that was opposite the one seen in the GSI. The K of large females was low from November (1.82 [SD 0.1]) to January (1.80 [SD 0.0]) and increased from February (1.82 [SD 0.0]) to July (1.89 [SD 0.2]) (Fig. 4A). Except for January, the mean HSI values showed a similar pattern to the one observed in the GSI. The HSI values of females >100 cm FL decreased from February (1.27 [SD 0.3]) to April (0.61 [SD 0.1]) and then increased slightly from May (0.70 [SD 0.1]) to July (0.72 [SD 0.2]) (Fig. 4A). The data series for the HSI was shorter than the data series for the other 2 condition indices because no liver samples were collected in the cannery.

Oocyte developmental stage and condition indices

There were significant differences in the GSI (H_{275}=162.1, P<0.01), HSI (H_{275}=49.9, P<0.01), and K (H_{275}=10.68, P<0.05) between ovarian developmental phases. The GSI showed lowest values at the primary growth stage (0.23 [SD 0.1]) and values increased throughout the vitellogenesis process (1.13 [SD 0.8]) until the maximum values at GVM (2.17 [SD 0.7]) and hydration (1.98 [SD 0.8]) stages were reached. Afterward, the GSI showed a sharp decrease in the regenerating phase (0.44 [SD 0.3]) (Fig. 5). The HSI showed a decreasing trend from immature phase ovaries (0.94 [SD 0.2]) to ovaries in vitellogenesis (0.77 [SD 0.2]), and then it followed the pattern shown by the GSI, increasing from vitellogenesis to hydration stages (1.33 [SD 0.3]). The HSI had its minimum value in the regenerating phase (0.66 [SD 0.1]). The K followed the opposite trend from that of the GSI and HSI; it decreased from the immature phase (1.94 [SD 0.2]) throughout the maturation process, obtaining minimum values at the hydration stage (1.77 [SD 0.0]). The K increased in the regenerating phase (1.85 [SD 0.1]).

Fecundity Estimation

The estimated mean BF was 3.07 million oocytes and varied from 0.32 million to 6.91 million oocytes. The estimated mean BFrel was 74.4 oocytes per gram of gonad-free weight and fluctuated between 9.2 and 180.8 oocytes per gram of gonad-free weight. Batch fecundity

Table 3

Summary of oocyte developmental stages and corresponding reproductive phases by 5-cm size class (fork lengths) in our study of Yellowfin Tuna (*Thunnus albacares*) reproduction in the western Indian Ocean. The numbers of individuals in our study that were at each developmental stage are shown in the columns organized by histological classification of the ovaries (Brown-Peterson et al. 2011; Zudaire et al., 2013): immature phase, including the primary growth stage (PG); developing phase, including the cortical alveolar (CA), primary vitellogenesis (Vtg1), and secondary vitellogenesis (Vtg2) stages; spawning-capable phase, including the tertiary vitellogenesis (Vtg3), germinal vesicle migration (GVM), and hydration stages (Hydr.); and regenerating phase.

		Mature phases and developmental stages							
	Immature phase	Developing to spawning-capable							
Length (cm)	PG	CA	Vtg 1	Vtg 2	Vtg 3	GVM	Hydr.	Regenerating	Total
48–53	15	2							17
53–58	45	6							51
58–63	51	3							54
63–68	12	5							17
68–73	15	8	1	1					25
73–78	19	10	5	1	2			1	38
78–83	26	51	8	7	4	1		8	105
83–88	25	42	17	5	6	2		10	107
88–93	6	32	16		3			1	58
93–98	4	28	16	12	4	2		4	70
98–103	7	14	7	3	5	1		1	38
103–108	17	12	6	4	2			10	51
108–113	4	14	1		1			6	26
113–118	1	3	1	1	6				12
118–123		3			13	2		1	19
123–128				1	12	5	1		19
128–133			1	3	20	11	1		36
133–138			1	2	21	16	1		41
138–143		4	1	3	11	5	1		25
143–148		1		1	2	3	1		8
148–153			1				1		2
Total		238	82	44	112	48	6	42	819
	247				572				

was positively related to GSI, length, and weight; BFrel was related only to GSI; HSI and K showed no significant relationship with BF or BFrel (Table 4). The GSI showed the best correlation with BF and BFrel (coefficient of correlation [r]=0.87 and r=0.89, respectively). In contrast, low values for r^2 were found for length and weight (0.25). The intercept of the regression line between the BF and fish weight relationship was significantly different from 0.00 (P<0.05), a result that could indicate that the number of oocytes produced per gram of female is not linearly related to weight, and, therefore, larger females produce more oocytes per gram of gonad-free weight than do smaller females (Fig. 6). A decrease in mean BF values from November (3.79 million) to June (2.26 million) was observed. Nevertheless, the ANOVA of the BF estimates by month did not reveal statistically significant relationships between months at a 95% confidence level (ANOVA; $F_{(5,42)}$=1.52, P=0.2081). The BF appeared to be highly variable by month, making it difficult to identify a clear pattern for female fecundity.

Discussion

Length at 50% maturity

Analyses of L_{50} for female Yellowfin Tuna in the Indian Ocean in other studies resulted in different estimates (Table 5). To our knowledge, our study is only the second one where the L_{50} of Yellowfin Tuna in the Indian Ocean has been examined by histological method. Timochina and Romanov[3] previously used a histological

[3] Timochina O. I., and E. V. Romanov. 1991. Notes on reproductive biology of Yellowfin tuna in the western Indian Ocean. IPTP (Indo-Pacific Tuna Development and Management Program), Coll. Vol. Work. Doc. TWS/91/32, 60 p. P.O. Box 2004, Colombo, Sri Lanka.

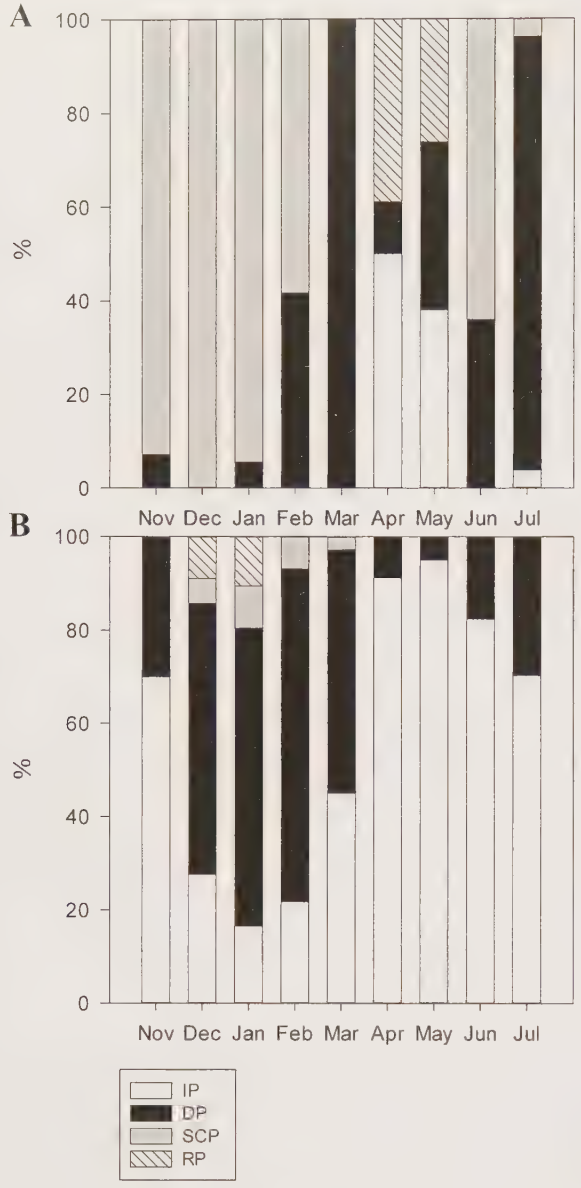

Figure 3

Proportion of female Yellowfin Tuna (*Thunnus albacares*) in different ovarian developmental phases by month and size: (**A**) >100 cm in fork length and (**B**) <100 cm in fork length. The phases of ovarian developmental shown in this graph are the immature phase (IP), developing phase (DP), spawning-capable phase (SCP), and regenerating phase (RP).

method to estimate the length at which all specimens achieve maturity. In our recent work, L_{50} was estimated at 75 cm FL when the maturity threshold was defined as ovaries in the CA stage. This length is significantly smaller than the L_{50} reported for all previous studies in the Indian Ocean (Table 5). In all of those studies, the L_{50} was estimated on the basis of macroscopic methods that defined the maturity threshold by the presence of advanced vitellogenic oocytes in the ovaries. Besides the natural variability in the length at maturity (Itano[2]), the main reasons for the difference in L_{50} between our study and previous studies are the use of different methods and oocyte stages to establish the maturity threshold for estimating L_{50}. Standardization of the selected maturity index and accurate estimation are required to enable direct comparisons between estimates and to avoid biases.

The cortical alveolar stage is the earliest sign of oocyte maturation (Brown-Peterson et al., 2011). Females in this developmental stage normally continue through vitellogenesis and spawn in the upcoming season (Wright, 2007). On the basis of the annual reproductive cycle and batch spawning behavior of Yellowfin Tuna, following the recommendation made by Lowerre-Barbieri et al. (2011), we suggest that Yellowfin Tuna females with ovaries in the cortical alveolar stage should be included in maturity estimates. Estimation of L_{50} through establishment of maturity in vitellogenic oocytes (Schaefer, 1998; Itano[2]; Zhu et al., 2008) has the disadvantage that L_{50} will be overestimated because maturing individuals (i.e., females with cortical alveolar stage oocytes) are categorized as immature (Lowerre-Barbieri et al., 2011). There is no information on how much individual growth occurs during the time lag between CA and vitellogenesis. Such information may improve estimation of L_{50} because growth between those stages may partly explain the difference in length at first maturity obtained with different oocyte maturation thresholds.

Reproductive cycle

On the basis of the histological evaluation of ovaries and the assessment of the seasonal variation in the GSI, 2 main reproductive periods were identified in our study. The first period identified occurred from November to February, and the second period occurred in June, with lower reproductive activity than the first period. Similar results were obtained by Stéquert et al. (2001), who related the spawning activity of Yellowfin Tuna with the monsoons. They identified that spawning activity was higher in the north monsoon (from November to March) than in the south monsoon (from June to August)—probably a result of the decrease in sea-surface temperature during the south monsoon period. Other authors identified only a single reproductive period: from January to June (Zhu et al., 2008), from January to March (Stéquert and Marsac, 1989), and from November to April (Nootmorn et al.[4]). The seasonal peaks in spawning described in this study were

[4] Nootmorn, P., A. Yakoh, and K. Kawises. 2005. Reproductive biology of yellowfin tuna in the eastern Indian Ocean. Indian Ocean Tuna Commission (IOTC),Working Party Tropical Tuna (WPTT), IOTC-WPTT-14, 378–385 p. IOTC Secretariat, P.O. Box 1011, Victoria, Seychelles.

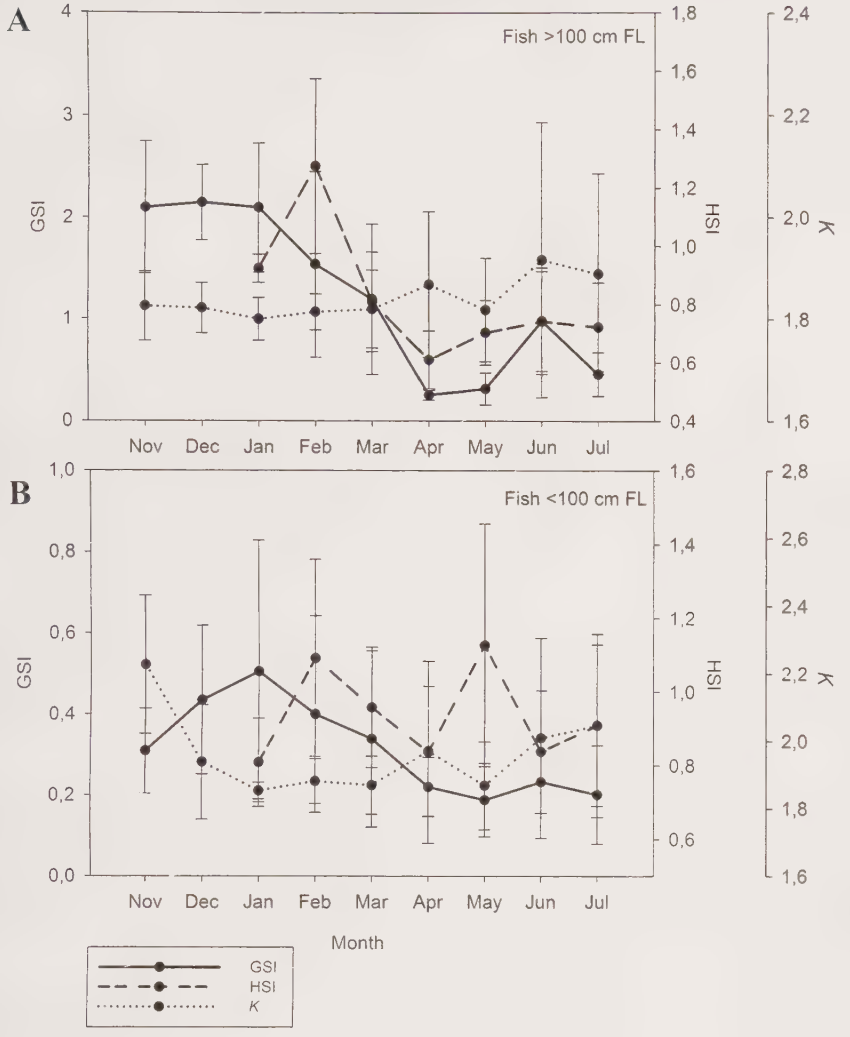

Figure 4

Mean monthly variation in the gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (*K*) in our study of the reproductive potential of Yellowfin Tuna (*Thunnus albacares*) in the western Indian Ocean for females in two groups: (**A**) >100 cm in fork length (FL) and (**B**) <100 cm FL

supported by GSI values over 1.5, the value corresponding to that fish capable of reproducing (Nootmorn et al.[4]), observed from November to January. The GSI values for February and June below 1.5 could correspond to a period of lower spawning activity, in which the proportion of active females among the mature population decreased. The absence of samples from August to October affected the interpretation of the reproductive cycle (e.g., the commencement of the main spawning period).

The analysis of the reproductive cycle by size groups showed asynchrony in the reproductive activity between small and large Yellowfin Tuna. Larger and older individuals spawned earlier and longer in the season and had a higher activity than smaller individuals. This spawning behavior is in accordance with McPherson (1991) and Schaefer (1998), who described a positive relationship between the spawning fraction and female size. Our results showed asynchrony in the appearance of ovaries in the regenerating phase between small and large fish. Smaller individuals showed high percentages of ovaries in this phase earlier (December and January) than did large specimens (April and May). Ovaries of smaller individuals appeared in the regenerating phase within a period (from November to February) in which females generally exhibit high reproductive activity. This behavior can occur more often among young individuals maturing for the first time than among older fish (Murua et al., 2003), and it may be related to the energy balance between somatic growth and reproduction, an energy balance that is size related (Claramunt et al., 2007).

Another hypothesis that may explain the asynchrony in reproductive timing among sizes is that young females skip spawning events. The early appearance of ovaries in the regenerating phase occurred during the main peak of reproductive activity of mature individuals, and it is unlikely to be related to the end of the spawning season of young fish (Murua and Saborido-Rey, 2003). Female Yellowfin Tuna that skip spawning can be classified as *younger skipping* females (Secor, 2007). In these first-maturing females, maturation involves large physiological and behavioral transitions. They may not have the required energy resources and, therefore, forego reproduction. By skipping spawning, these females increase their growth rate and their chances of survival, resulting in increased lifetimes and reproductive outputs (Rideout and Tomkiewicz, 2011). However, the extended spawning season of Yellowfin Tuna, as well as the variable spawning period among individuals, complicates the identification of skipped spawning seasons in the reproductive cycle of females (Lowerre-Barbieri et al., 2009). Therefore, further research is needed to

Table 4

Coefficients of determination (r^2) and correlation (r) and P-values (P) for the relationship between batch fecundity (BF) and relative batch fecundity (BFrel) with different biological parameters: fork length, weight, gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (K) in our study of Yellowfin Tuna (*Thunnus albacares*) reproduction in the western Indian Ocean.

	r^2	r	P
BF			
Length (cm)	0.2114	0.4598	<0.005
Weight (g)	0.2022	0.4497	<0.005
GSI	0.7579	0.8706	<0.001
HSI	0.0237	0.1541	0.6706
K	0.0611	−0.2472	0.1145
BFrel			
Length (cm)	0.0377	0.1943	0.2174
Weight (g)	0.0263	0.1622	0.3046
GSI	0.8099	0.8999	<0.001
HSI	0.0696	0.2639	0.4612
K	0.0184	−0.1357	0.3914

determine whether the appearance of regenerating phase ovaries among young individuals indicates that females have skipped the spawning season.

Condition indices related to reproduction

Condition indices are important parameters for tuning the estimation of reproductive potential (Marshall et al., 1999). Analysis with such indices throughout the spawning season allows the determination of energy allocation during reproduction (Murua and Motos, 2006). In our study, during the peak spawning period (November–February), GSI, HSI, and K reflected a seasonal pattern in the accumulation and depletion cycles of energy reserves. The increase in the GSI and HSI at the expense of K was observed both in smaller (<100 cm FL) and larger (>100 cm FL) females, and the exchange of energy was more pronounced in the larger size group. These 3 condition indices from March to July showed higher variability than in the first spawning period (from November to February), and no distinct pattern was evident. For the period of higher spawning activity (November–February), condition indices showed a clear pattern of energy mobilization from the muscle to the liver or gonad for reproduction. In contrast, during the second spawning period (June), energy was mobilized at a lower level because of lower spawning activity.

The protracted spawning season and population asynchrony in spawning activity of Yellowfin Tuna could mask temporal variations in energy allocation and the mobilization of the factors that were analyzed (Domínguez-Petit et al., 2010). Therefore, the assessment of variation in energy reserves by maturation phases was performed to study the energy cycle in females undergoing ovarian development (Alonso-Fernández and Saborido-Rey, 2012). The results showed a high energy investment in reproduction. The GSI showed an increase in the ovary mass from the immature phase to hydration stage, and the HSI described an increase of liver mass mainly between the vitellogenic process and maturation. This HSI pattern is evidence of the importance of the liver in the energy accumulation and synthesis of those energetic compounds (e.g., lipids and vitellogenin) essential for ovarian development (Domínguez-Petit et al., 2010). The decrease of K during the vitellogenic process and the low values of K at the GVM and hydration stages could indicate the role of the muscle in the mobilization of energy to the gonad or liver to fulfill the energetic requirements of maturation, principally during vitellogenesis (Zaboukas et al., 2006; Domínguez-Petit et al., 2010).

Table 5

Estimates of the length at 50% maturity (L_{50}) and the fork length at which all specimens achieve maturity (L_{100}) for female Yellowfin Tuna (*Thunnus albacares*) reported for previous studies in different areas of the Indian Ocean. The methods applied for the classification of ovaries were macroscopic (Macro.) and microscopic (i.e., histological, Micro.). The criterion for the maturity threshold in all of these studies was the presence of vitellogenic stage oocytes.

Studies	Estimation type	Method	Length (cm)
Stéquert and Marsac, 1989	L_{50}	Macro.	120–140
Hassani and Stéquert (*see fn. 5 in the text*)	L_{50}	Macro.	110–115
Nootmorn et al. (*see fn. 4 in the text*)	L_{50}	Macro.	110
Karpinski and Hallier[7]	L_{50}	Macro.	104–112
Zhu et al. 2008	L_{50}	Macro.	114
Timochina and Romanov (*see fn. 3 in the text*)	L_{100}	Micro.	120
Maldeniya and Joseph[8]	L_{50}	Macro.	100

[7] Karpinski, B., and J. P. Hallier. 1988. Preliminary results on yellowfin spawning in the western Indian Ocean. Indo-Pacific Tuna Development and Management Program (IPTP) Coll. Vol. Work. Doc.TWS/88/31, 50–59 p.

[8] Maldeniya, R., and L. Joseph. 1986. On the distribution and biology of yellowfin tuna (*T. albacares)* from the western and southern coastal waters of Sri Lanka. FAO/IPTP Coll. Vol. Work. Doc. 2: TWS/86/18, 21–32 p.

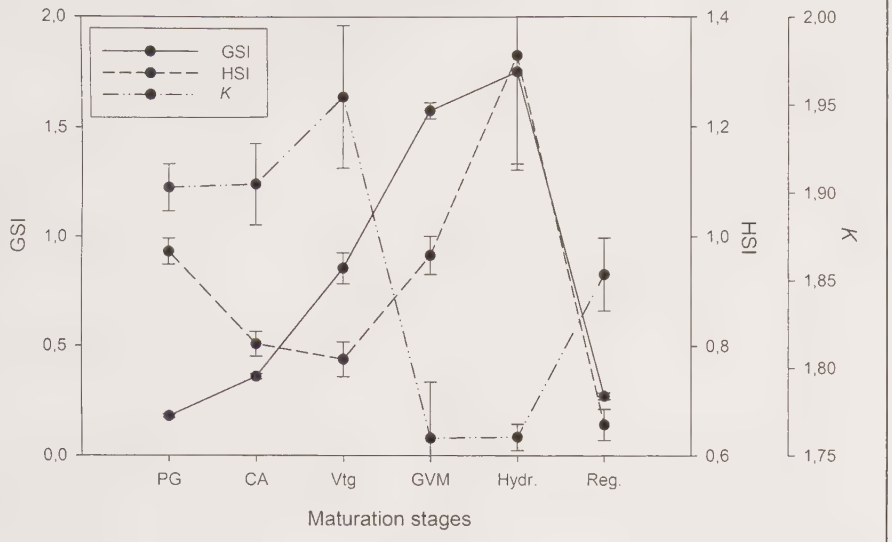

Figure 5

Mean variation in gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (*K*) for Yellowfin Tuna (*Thunnus albacares*), in our study of the reproductive potential of this species in the western Indian Ocean, by oocyte developmental stage: primary growth (PG), cortical alveolar (CA), primary, secondary, and tertiary vitellogenesis (Vtg); germinal vesicle migration (GVM); hydration (Hydr.), and regenerating (Reg) stage.

The energy allocation strategy varies among organisms as an adaptive measure to deal with the fluctuations in the availability of energy in marine environments (Alonso-Fernández and Saborido-Rey, 2012). Subtropical and tropical waters with relatively low fluctuations in environmental conditions (e.g., in food supply, temperature, and photoperiod) are conducive habitats for species to offset the cost of the reproductive process by concurrent energy from feeding, without having to rely entirely on stored energy reserves (Alonso-Fernández and Saborido-Rey, 2012). Yellowfin Tuna continue to feed while they reproduce, and it has been suggested that the high spawning activity of this species depends on prey availability during spawning (Itano[2]). On the basis of our results, we suggest that Yellowfin Tuna, like other tropical species (Arrington et al., 2006), requires energy from feeding as well as from stored energy to carry out ovarian development. Therefore, Yellowfin Tuna could be described as a capital-income breeder (Alonso-Fernández and Saborido-Rey, 2012), in which the energy stored before reproduction is not enough to offset the cost of reproduction, and energy allocation from feeding is necessary for successful reproduction (Henderson and Morgan, 2002).

Fecundity estimation

Few studies have dealt with the fecundity of Yellowfin Tuna in the Indian Ocean. In our study, the estimated mean BF (3.1 million oocytes) is within the range reported by Hassani and Stéquert,[5] and in Table 6 by Timochina and Romanov[2] for the western Indian Ocean and by Sun et al.[6] for the western Pacific Ocean. However, our estimate is larger than the values reported by Schaefer (1996, 1998) for the eastern Pacific Ocean and by Itano[2] for the western Pacific Ocean. It is lower than the values reported by Itano[2] for the Hawaii area. Furthermore, the mean BFrel value (74.4 oocytes per gram of gonad-free weight) determined in our study for Yellowfin Tuna is slightly higher than the values described by Schaefer (1996, 1998) and Sun et al.[6] (Table 6), and it is considerably higher than the values reported by Itano[2]. Besides the geographic differences among studies, intrapopulation variability in fecundity (Schaefer, 1996) could be the main factor that caused the difference between the results of our study and previously published results.

The BF increased with female size and weight ($P<0.05$); however, the BF-length and BF-weight relationships showed low r^2 (<0.25) with high variability—a finding that could result from the asynchrony of the population spawning (i.e., with some individuals at the beginning and others at the end of their individual spawning season). The intercept of the regression line between the BF and weight was statistically different from zero, indicating that larger females spawn more oocytes per gram than smaller females; this dynamic in turn would contribute to increasing the egg production for large females (Domínguez-Petit and Saborido-Rey, 2010). However, the BF of fish of the same size varied greatly, indicating that other factors, such as fish condition during spawning, could also drive female productivity (Hassani and Stéquert[5]; Murua and Motos, 2006).

Experiments with captive Yellowfin Tuna showed evidence of a positive relationship between the daily food ratio and egg batch production (Margulies et al.,

[5] Hassani S., and B. Stéquert. 1990. Sexual maturity, spawning and fecundity of the yellowfin tuna (*Thunnus albacares*) of the Western Indian Ocean. Coll. Vol. IPTP (Indo-Pacific Tuna Development and Management Program) Doc. Vol. 4, p. 91–107. IPTP, P.O. Box 2004, Colombo, Sri Lanka.

[6] Sun, C., W. Wang, and S. Yeh. 2005. Reproductive biology of yellowfin tuna in the central and western Pacific Ocean. Western and Central Pacific Fisheries Commission (WCPFC). WCPFC-SC1, BI WP-1, 1–14 p.

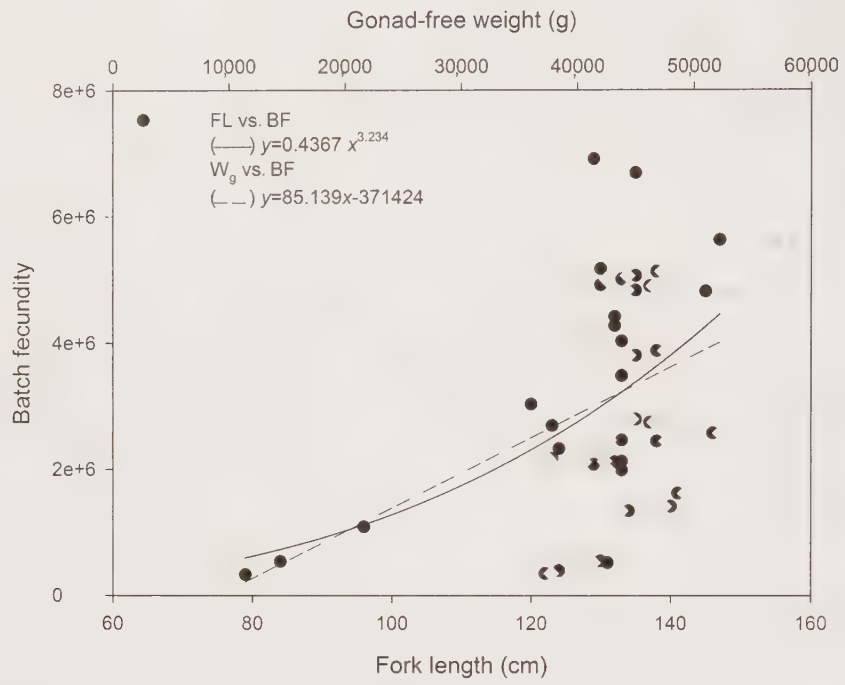

Figure 6

Linear regression between batch fecundity (BF) and fish gonad-free weight (W_g), and power function between BF and fork length (FL) for Yellowfin Tuna (*Thunnus albacares*) sampled in the western Indian Ocean in 2009 and 2010

2007). Lauth and Olson (1996) suggested that tuna may boost batch fecundity in response to greater amounts of forage, in accordance with the results reported by Itano[2] for the Pacific Yellowfin Tuna. Batch fecundity enhancement by feeding might explain the observed high interindividual variation in the BF. Apart from prey availability, other biotic and abiotic parameters, such as fish condition, genetic variation, geographic differences in sampling locations, and temperature, have been reported to be associated with variability in the productivity of fish species (Schaeffer, 1998; Murua and Motos, 2006).

Conclusions

Advances in the management of fish stocks rely to a great extent on updated knowledge of reproductive dynamics and its implementation in stock assessments. The results described here will contribute to the improvement of the understanding of the reproductive dynamics of Indian Ocean Yellowfin Tuna. The L_{50} was estimated at 75 cm FL with the maturity threshold set at the CA stage of ovarian development. Two reproductive periods were detected in which reproductive activity was higher in large females than in small ones. Similarly, large females had higher contribution of fecundity than did small females; however, more research on fecundity is required to investigate the principal factors that affect its variability at individual and population levels. We suggest that Yellowfin Tuna follow a capital-income breeder strategy during ovarian development, by mobilizing accumulated energy and incorporating energy from feeding. However, further research on the proximate composition (e.g., lipids and proteins) of this species through analyses of different tissues is required to obtain more precise descriptions of energy allocation and mobilization during the reproductive cycle of Yellowfin Tuna.

Table 6

Estimates of the batch fecundity (BF) and the relative batch fecundity (BFrel) of Yellowfin Tuna (*Thunnus albacares*) reported for previous studies in different areas of Pacific and Indian Oceans. Values are expressed in millions for BF and in oocytes per gram of gonad-free weight for BFrel.

Studies	Area	BF	BFrel
Hassani and Stéquert (*see fn. 5 in the text*)	Western Indian Ocean	0.50–8.00	–
Timochina and Romanov (*see fn. 3 in the text*)	Western Indian Ocean	3.27	–
Schaefer 1996	Eastern Pacific Ocean	1.57	68.0
Schaefer 1998	Eastern Pacific Ocean	2.50	67.3
Itano (*see fn. 2 in the text*)	Hawaii area	3.45	63.5
Itano[2]	Equatorial western Pacific	2.16	54.7
Sun et al. (*see fn. 6 in the text*)	Western Pacific Ocean	2.71	62.1

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Age, size, and sexual maturity of channeled whelk (*Busycotypus canaliculatus*) in Buzzards Bay, Massachusetts

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Abstract—With the southern New England lobster fishery in distress, lobster fishermen have focused more effort toward harvesting channeled whelk (*Busycotypus canaliculatus*). However, minimal research has been conducted on the life history and growth rates of channeled whelk. Melongenid whelks generally grow slowly and mature late in life, a characteristic that can make them vulnerable to overfishing as fishing pressure increases. We sampled channeled whelk from Buzzards Bay, Massachusetts, in August 2010 and in July 2011, studied their gonad development by histology, and aged them by examining opercula. Males had a slower growth rate and a lower maximum size than females. Male whelk reached 50% maturity (SM_{50}) at 115.5 mm shell length (SL) and at the age of 6.9 years. Female whelk reached SM_{50} at 155.3 mm SL and at the age of 8.6 years. With a minimum size limit of 69.9 mm (2.75 in) in shell width, males entered the fishery at 7.5 years, a few months after SM_{50}, but females entered the fishery at 6.3 years, approximately 2 years before SM_{50}. Increased fishing pressure combined with slow growth rates and the inability to reproduce before being harvested can easily constrain the long-term viability of the channeled whelk fishery in Massachusetts.

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The channeled whelk (*Busycotypus canaliculatus*: Melongenidae) supports a small but growing fishery in Massachusetts. Most fishing is conducted by lobstermen during the off-season (spring and fall) or when the lobster fishery is slow; therefore, fishing of this species typically is done on a part-time basis. However, channeled whelk landings in Massachusetts increased substantially after 2000, as the southern New England lobster stock declined, and reached 1400 metric tons in 2011 with a value of $6.2 million (Glenn and Wilcox[1]). In addition, exvessel prices have nearly doubled from 2007 to 2011, increasing the incentive to expand effort in this fishery (Glenn and Wilcox[1]). Fishing pressure may affect the average size of whelks because many fishermen may focus on catching larger whelks (>160 mm shell length [SL]). Davis and Sisson (1988) reported declines in population density and mean shell width (maximum distance across shell) for channeled whelk in Nantucket Sound between 1978 and 1981. Bruce (2006) reported a decrease in mean SL between 1994 and 2004 for a related species, knobbed whelk (*Busycon carica*), subject to a dredge fishery in Delaware Bay.

Most whelk research has been conducted on knobbed whelk, and minimal research has been done on channeled whelk (Avise et al., 2004; Bruce, 2006; Castagna and Kraeuter, 1994; Eversole et al., 2008; Kraeuter et al., 1989; Power et al.[2]; Walker et al., 2005; Walker et al., 2007). Because of limited information on growth rates and size at maturity, managers do not know if the current minimum size limit of 69.9 mm (2.75 in) in shell width (SW) is appropriate to ensure the reproduction and longevity of channeled whelk in Mas-

[1] Glenn, R., and S. Wilcox. 2012. Profile of the channeled whelk pot fishery, 9 p. Report to the Massachusetts Marine Advisory Commission. Massachusetts Division of Marine Fisheries, Invertebrate Fisheries Program, 1213 Purchase St., New Bedford, MA 02740.

[2] Power, A. J., C. J. Sellers, and R. L. Walker. 2009. Growth and sexual maturity of the knobbed whelk, *Busycon carica* (Gmelin, 1791), from a commercially harvested population in coastal Georgia, 24 p. Occasional Papers of the University of Georgia Marine Extension Service, vol. 4. Marine Extension Service, Univ. Georgia, Shellfish Research Laboratory, Savannah, GA.

sachusetts and to sustain the fishery for this species. The minimum size limit was established by the State of Massachusetts on the basis of the size of market acceptability (Glenn and Wilcox[1]). With increased fishing pressure and limited biological information, channeled whelk can easily become overfished, especially if they are not able to reproduce before they enter the fishery.

The channeled whelk ranges from Cape Cod, Massachusetts, to Cape Canaveral, Florida (Edwards and Harasewych, 1988). Growth rate and size at maturity for channeled whelk are virtually unknown, but whelks of the family Melongenidae typically are slow growing, late maturing animals. In the seaside lagoons of Virginia, knobbed whelk reach a mean size of 176.1 mm SL in 9–11 years (Kraeuter et al., 1989). In South Carolina, knobbed whelk <90 mm SL grow faster than larger whelk (up to 7 times faster), although some knobbed whelk have minimal or negative growth (Eversole et al., 2008). It has been suggested that channeled whelk have low fecundity because they lay egg strings only once a year (Edwards and Harasewych, 1988). Betzer and Pilson (1974) reported an annual change in gonad index (fresh weight of the gonad/fresh weight of whole soft tissues) of channeled whelk in Narragansett Bay, Rhode Island, with spawning most likely occurring in late summer and fall. No studies have been published that provide the spawning season of channeled whelk or the environmental factors, such as temperature and salinity, at which they spawn. In a study of this species in aquaria, channeled whelk began hatching from an egg string on 18–30 April 2010 at water temperatures of 15–18°C; the egg string was collected on 1 March 2010 near Cedar Island, Virginia (Harding, 2011).

Channeled whelk may have a similar reproductive cycle to that of knobbed whelk. On intertidal flats in Virginia, knobbed whelk copulated in June and July and laid egg strings from mid-August to November; hatching occurred from mid-March to early May (Castagna and Kraeuter, 1994). Knobbed whelk egg cases found in Cedar Island, Virginia, in 1977 yielded an average of 3770 whelk per string (Castagna and Kraeuter, 1994). However, information on the fecundity of channeled whelk is needed. The mode of reproduction for whelks also needs investigation because Castagna and Kraeuter (1994) suggested that knobbed whelk may be protandrous hermaphrodites. Knobbed whelk raised in a laboratory were all males at 9 years, but, after 13 years, some males changed sex, and at the age of 14 years, produced viable offspring (Castagna and Kraeuter, 1994). This outcome is contrary to the findings of Avise et al. (2004), who determined that knobbed whelk are genetically dioecious and sex is determined at birth.

Aging opercula gives insight on the growth and longevity of channeled whelk. Ilano et al. (2004) reported that striae form annually on the operculum of *Buccinum isaotakii* and can be used to estimate age. Heude-Bertherlin et al. (2011) counted the number of striae on the operculum of waved whelk (*Buccinum undatum*) to determine age. Kraeuter et al. (1989) aged knobbed whelk by embedding opercula in plastic resin and then sectioning them. For validation of aging, they used laboratory-reared knobbed whelk and embedded the opercula of 3 knobbed whelk of 6+ years and 3 knobbed whelk of 7+ years. Kraeuter et al. (1989) reported the average ages of these knobbed whelk at 6.0 and 7.2 years, respectively. Another aging technique involves bleaching knobbed whelk opercula and counting annuli (Bruce et al.[3]). In the opercula of older knobbed whelk, Power et al.[2] found a "bubbling effect," from growth ring overlap, due to decreased growth rates.

There are no published reports on the histological staging of channeled or knobbed whelk gonads. We began this study to provide useful biological information, such as size and age at sexual maturity, for managers of the channeled whelk fishery in Massachusetts. Data on the size at sexual maturity will provide managers with information needed to set minimum size limits that allow females to spawn at least once and, therefore, to help prevent overfishing (Gordon, 1994). We sampled channeled whelk in Buzzards Bay, Massachusetts, and expected that they would be protandrous hermaphrodites as reported for laboratory-reared knobbed whelk by Castagna and Kraeuter (1994). We hypothesized that SL would increase with age and that slower growth would occur at older ages. We provide the first information on the relationship between size, age, and stages of gonad development for channeled whelk and on the size and age at 50% maturity (SM_{50}) of male and female channeled whelk.

Materials and methods

Sampling

Sampling was conducted off Massachusetts in Buzzards Bay, a large, semi-enclosed estuary in the northeastern United States (Fig. 1). Buzzards Bay is uniformly shallow, with depths mostly at 10–15 m, and is open to the sea at multiple locations. As a result, the water column in this bay is extremely well mixed, and differences between surface and bottom temperatures rarely exceed 1°C, and salinities are almost uniformly 30 ppt (Turner et al., 2009). Commercial wooden or wire mesh conch traps, baited with the Atlantic horseshoe crab (*Limulus polyphemus*), were used to collect channeled whelk from Buzzards Bay in August 2010 and in July 2011. Traps varied in size but were generally 50×50×30 cm, and they were set at depths of 10–15 m at 10–12 different sites in each year to maximize catches. Traps were allowed to soak for 1 week, and they were retrieved weekly over a 4-week period each year. Channeled whelk were identified according to Pollock

[3] Bruce, D. G., R. Wong, and M. Greco. 2006. Delaware Bay whelk (conch) fishery assessment 2005, 36 p. Delaware Division of Fish and Wildlife, 89 Kings Highway, Dover, DE 19901.

(1998). Whelk from different sites were mixed; sites sampled in 2010 were mostly in eastern Buzzards Bay, and those sites sampled in 2011 were mostly in western Buzzards Bay. Because we mixed whelk from a large number of sites, we did not measure water temperature at individual sites; instead, seawater temperatures were examined from the NOAA Data Buoy Station BZBM3, at the Northeast Fisheries Science Center dock in Woods Hole, Massachusetts (NOAA station 8447930, http://www.ndbc.noaa.gov/station_history.php?station=bzbm3).

We measured whole wet weight (Wt) of shell and tissue to the nearest 0.1 g, SL to the nearest 0.1 mm, and lip width (LW, maximum distance across the upper edge of the largest whorl) to the nearest 0.1 mm. Length of the male copulatory organ (penis length), measured to the nearest 0.1 mm, was recorded for 93 males only in 2011. We also measured the SW for the first 166 whelk. Although SW is very similar to the measurement used by fishermen and managers for the minimum size limit, channeled whelk have an asymmetrical shell structure, and there is no standard procedure for measuring SW. We used LW instead of SW as our primary width measurement because it was more easily replicated and more precise than SW; the mean coefficient of variation for whelk in 5-mm intervals of LW was 2.14 times greater for SW than for LW, with a range from 1.21 to 4.13 for SW.

For this study, 292 channeled whelk (155 females and 137 males) were sampled through the random selection of at least 20 whelk of various sizes in each 10-mm-SL interval. In 2010, we filled most of our SL intervals except for the intervals for very small (<100 mm SL) and very large (>190 mm SL) whelk. In 2011, we focused on the collection of whelk from these unfilled SL intervals to extend the sampling range for this study. After whelk were weighed and measured, we cracked their shells with a hammer to determine sex. Males were defined as those whelk with a penis, and females were defined as those whelk with a nidamental gland. Scissors were used to separate the gonad from the digestive gland (gonads were not weighed). We removed the entire gonad from smaller whelk (<120 mm SL), and larger whelk had an approximately 2-cm section removed from the terminal end of the gonad (closest to the tip of the shell spire) to the start of the gonad (closest to the operculum). Gonads were stored in 10% formalin for 1 week before they were transferred to 80% ethanol (EtOH) (Stoner et al., 2012).

Figure 1

Map of approximate capture sites (gray circles) of channeled whelk (*Busycotypus canaliculatus*) collected in August 2010 and July 2011 in Buzzards Bay, Massachusetts. Specimens came from numerous sites within Buzzards Bay; however, capture site was not recorded for each whelk.

Age analysis

Opercula from our dissected channeled whelk were removed with a scalpel, labeled, and stored in 10% formalin for 1 week, and then transferred to 70% EtOH. Whelk opercula were taken out of EtOH, bleached for 30 min with 5% sodium hypochlorite, and then blotted dry with paper towels. Annuli were defined as growth striae that form on the interior margin and extend across the operculum to the exterior margin (Fig. 2; Bruce et al.[3]). The first annulus of each operculum generally had a 6-mm linear distance that ranged from the interior to the exterior margin. To count annuli, opercula were placed under a light source and photographed with an Olympus Stylus 400[4] 4-megapixel digital camera (Olympus Optical Co., Ltd., Center Valley, PA). Opercula did not dry and curl during this pro-

[4] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

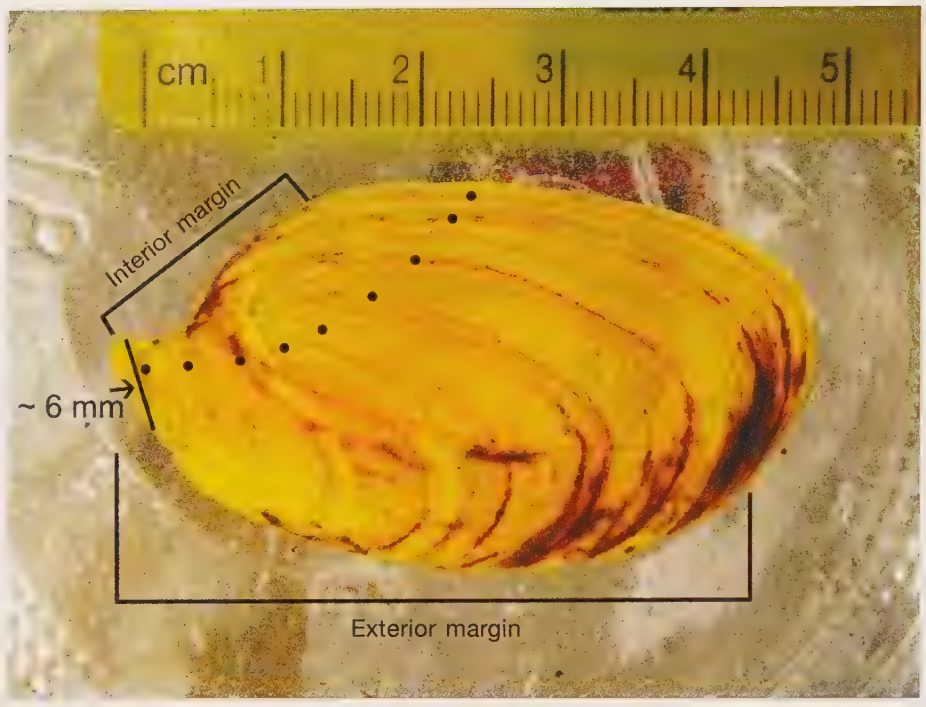

Figure 2

Image of the operculum of a 9-year-old male (142.9 mm shell length) channeled whelk (*Busycotypus canaliculatus*). Readers counted annuli to determine the age of whelk collected for our study in August 2010 and July 2011 in Buzzards Bay, Massachusetts. Dots are placed on each annulus for reference. The interior margin is at the top of this opercula, and the exterior margin is at the bottom. For the first annulus of each operculum examined in our study, there generally was a 6-mm linear distance from the interior to the exterior margin.

cess, and shrinkage was not a concern because counting of annuli did not depend on accurate measurement of widths of annuli.

Each operculum was read by 3 readers to estimate age (Kraeuter et al., 1989). Each reader recorded their confidence for each operculum, a technique similar to the one used by Herbst and Marsden (2011) to age otoliths for Lake Whitefish (*Coregonus clupeaformis*). Confidence levels for aging channeled whelk opercula were defined as 1) not confident—the initial year's growth was at least partially missing and annuli were difficult to distinguish because of opaque or dark zones on the operculum; 2) somewhat confident—the initial year's growth was partially missing but the shape was reasonably approximated; annuli were partially obscured by washed out or dark zones but were reasonably approximated; 3) confident—operculum showed initial year's growth and annuli were easily readable; or 4) very confident—operculum exhibited strongly prominent annuli that were easy to read.

We analyzed operculum only from whelk with an average confidence rating ≥2. If 2 of the 3 readers recorded the same age for an operculum, that age was used. If all 3 readers recorded different ages, the mean value (rounded to the nearest year) was used if variance was ≤1 (equivalent to a confidence bound of ±2 years); whelk with greater variance were determined to be unacceptable. Only 227 whelk (115 females and 112 males) of 292 total dissected whelk (155 females and 137 males) were used for operculum analysis (Table 1).

Histology and gonad staging

A histological examination of gonads from 112 males and 115 females was undertaken to determine gonad development stage. Not all dissected whelks (155 females and 137 males) were used for analysis because some opercula were not determined acceptable for operculum analysis and, therefore, could not be used for the comparison of age with gonad development. Two tissue samples were processed from each whelk gonad with a Tissue-Tek VIP-E150 (Sakura Fineteck USA, Inc., Torrence, CA) automated tissue processor that contained a dehydration series of 70% EtOH, 95% EtOH, and various concentrations of 100% EtOH and clearing agent and melted paraffin. Tissue samples were taken from a one-third portion and a two-thirds portion (approximately 5 mm apart) of the gonad sample to address synchrony within the gonad. The processed tissues were embedded in paraffin and sectioned to 10 µm (male) or 14 µm (females). Each tissue sample produced 4 replicates, which were mounted on slides and dried on a slide warmer. The best 2 slides from each tissue sample (4 slides per gonad) were stained with a haematoxylin and eosin stain; coverslips were then mounted with a resin mounting medium.

Four slides from each whelk gonad were examined to determine sex and gonad stage. The dominant stage was defined as the stage that represented at least 50% of the section (Ilano et al., 2003). If both tissue samples from a whelk's gonad had different dominant stages, then the most advanced stage was recorded. The classifications for each stage followed descriptions of *Buc-*

cinum isaotakii (e.g., Ilano et al., 2003) and *Rapana venosa* (e.g., Mann et al., 2006). Male gonads exhibited 4 stages: immature (I), early developing (ED), late developing (LD), and mature (M). Male maturity was classified on the basis of the presence of spermatozoa in the tubule and not on the basis of density. For classification in a fifth stage, the recovering (R) stage, males would be expected to exhibit elongate tubules with few remaining spermatozoa. No males were considered in the R stage because all males with elongate tubules had ≥50% of tubules with spermatozoa and were subsequently reported as in the mature stage. Female gonads exhibited 5 stages: I, ED, LD, M, and R. Female maturity was determined on the basis of the amount of vitellogenic oocytes (VOs) present in the cross section.

Statistical analyses

Linear regression relationships between SL, LW, and Wt were calculated and compared through the use of covariance analysis to determine if there was a relationship between sex and shell size or shape (Stoner et al., 2012). A significant coefficient ($P<0.05$) meant that either slope or intercept differed between males and females. As a prerequisite for covariance analysis, an F-test was used to ensure that the variance between males and females was not significantly different. Data were $\log_{10}$ transformed for the relationship between SL and Wt because Wt increased nonlinearly to SL. For the comparison of $\log_{10}$ *SL* and *sex* to $\log_{10}$ *Wt*, *sex* was a factor and $\log_{10}$ *SL* was a covariate.

The von Bertalanffy growth model has been used to describe the growth of gastropods, such as the queen conch (*Strombus gigas*) (e.g., Berg and Olsen, 1989) and *Buccinum isaotakii* (e.g., Ilano et al, 2004). Shell length and estimated age for each channeled whelk were used to fit a von Bertalanffy growth model, for each sex separately (Ricker, 1975):

$$L_a = L_{inf}\,(1 - e^{-K\,(a\,-\,t_0)}),$$

where L_a = the SL (in millimeters) at age (years);
L_{inf} = the theoretical maximum SL;
K = the growth coefficient;
a = the age; and
t_0 = the theoretical age at length 0.

A von Bertalanffy growth model that compared LW (in millimeters) and estimated age also was conducted, with LW replacing SL where appropriate. The annual growth rate (in SL) for each sex was calculated by subtracting the size at each age from the size at age+1 from the von Bertalanffy growth model. Growth rate and age were square-root (sqrt) transformed and covariance analysis was used to determine if sex affects growth. The sqrt transformation was used because a $\log_{10}$ transformation yielded nonlinear results. The regression of sqrt-transformed *SL growth* versus *age* and *sex* included *sex* as a factor and sqrt (*age*) as a covariate and was expressed (in terminology of R statistical software [R Development Core Team, 2011]) in this manner:

$$\text{sqrt}\,(SL\ growth) \sim \text{sqrt}\,(age) * sex,$$

where ~ indicates a relationship ("modeled as") and * indicates the combination of the factors sqrt (*age*) + *sex* and the interaction of sqrt (*age*) by *sex*. All further regression equations used this terminology and were computed with R statistical software (R Development Core Team, 2011).

On the basis of results of reproductive histology, we classified as mature any whelk with gonads in stage M or R. These data were used to calculate a nonlinear logistic regression by using a general linear model (GLM) with a binomial link function, with SL, LW, or age as the size measurement, and the GLM regression coefficients were used to estimate the size at which channeled whelk of each sex reached SM_{50} with the following formula:

$$SM_{50} = -B_0 \cdot B_1^{-1},$$

where B_0 = the intercept; and
B_1 = the slope.

Because the SM_{50} equation gives only a single value for each data set (male or female), a bootstrap routine was used to resample each data set with replacement 1000 times, and the results were used to calculate the bias and standard error (SE) of the original mean value of SM_{50}. Bias was calculated through the subtraction of the original (full data set) value of SM_{50} from the resampled mean value of SM_{50}. SM_{50} is a widely accepted predictor of sexual maturity for various shellfishes, including the queen conch (e.g., Stoner et al., 2012), waved whelk (e.g., Heude-Bertherlin et al., 2011), and *Zidona dufresnei* (e.g., Giménez and Penchaszadeh, 2003). In our study, the fitted values of the GLM, representing the proportion of mature whelk, were plotted against either SL or LW. For comparison, the proportion of mature whelk was calculated from the raw data within 10-mm increments of SL or 5-mm increments of LW and plotted. To compare the LW at which each sex reached SM_{50} with the current minimum size limit for harvest, we conducted a regression that compared the LW of 166 channeled whelk to their SW, the measurement similar to the one used to specify the legal size limit. All SM_{50} values are given as mean ±1 SE, and all statistics were computed with R (R Development Core Team, 2011).

Results

Staging gonads

Seawater temperatures at Woods Hole in the sampling months of August 2010 and July 2011 averaged 22.2°C (SE 0.7) and 22.2°C (SE 0.6), respectively, and were not significantly different, but mean temperatures in August were 1.2°C greater in 2011 than in 2010. To our

knowledge, there is no other published information about temperatures during the spawning season of channeled whelk, but our data do not allow inferences to be made about temperature effects on spawning because we sampled only during 1 month in each of 2 years and did not sample throughout the entire spawning season.

Histological examination showed that male channeled whelk classified as stage I of gonad development had small circular tubules that contained no spermatozoa and few spermatogonia, spermatocytes, and spermatids (Fig. 3A). The cross section contained connective tissue and empty space. Males assigned to stage ED had <25% of tubules with spermatozoa (Fig. 3B). There were few spermatogonia, spermatocytes, and spermatids present, and some empty space was still visible in the cross section. Males in stage LD had 25–50% of tubules with spermatozoa; more spermatogonia, spermatocytes, and spermatids were present and tubules were more elongate than they were in males in earlier stages (Fig. 3C). Males classified as stage M had ≥50% of tubules with spermatozoa; tubules were elongate and contained many spermatogonia, spermatocytes, and spermatids (Fig. 3D).

Female channeled whelk classified in development stage I had gonads with minimal previtellogenic oocytes (PVOs), no nuclei present, and empty space in the cross section; connective tissue was more prevalent than it was in later stages, as seen in males classified as stage I (Fig. 3E). Females in stage ED had more developed PVOs that contained round nuclei and visible nucleoli than did females in the earlier stage (Fig. 3F). Females identified as LD generally had <50% of the cross section with VOs (Fig. 3G). The VOs were larger and more elongate than the PVOs, although some PVOs still were present in the gonad. Females classified as M had ≥50% of the cross section with VOs, which were elongate and full of large yolk granules (Fig. 3H). Females in stage R had some elongate oocytes and some small oocytes that were empty or had minimal PVOs or VOs (Fig. 3I).

Morphological relationships

The ratio of females to males in our sample of dissected channeled whelk was near unity at the size range of 90–110 mm SL (Table 1) and ages of 4–6 years (Table 2). The smallest size intervals (70–90 mm SL) were male dominated, although not many individuals in that range were caught (Table 1). Male whelk had a lower maximum size and age than female

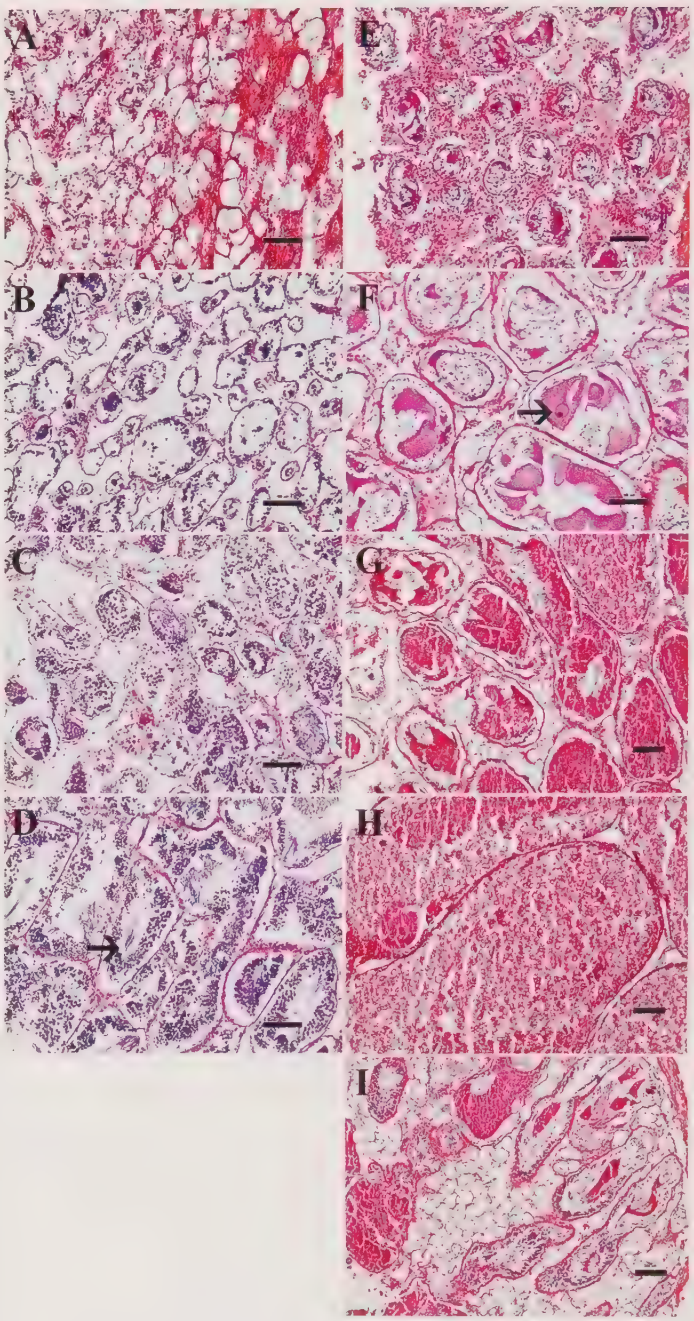

Figure 3

Histological photographs of the stages of gonad development for (**A–D**) male and (**E–I**) female channeled whelk (*Busycotypus canaliculatus*) used in our study to classify whelk collected in Buzzards Bay, Massachusetts, in July 2010 and August 2011. Observed male gonad stages: (**A**) immature; (**B**) early developing; (**C**) late developing; and (**D**) mature, with arrow pointing to spermatozoa. Observed female gonad stages: (**E**) immature; (**F**) early developing, with arrow pointing to a nucleus (also containing a nucleolus) from a previtellogenic oocyte; (**G**) late developing; (**H**) mature female with vitellogenic oocytes full of large yolk granules; and (**I**) recovering. Scale bars are set at 100 µm for panels A–F and 200 µm for panels G–I.

Table 1

Number and proportion of each sex by interval of shell length (SL) for channeled whelk (*Busycotypus canaliculatus*) collected in August 2010 and July 2011 in Buzzards Bay, Massachusetts. Lower and Upper SL describe the range for each interval and is measured in millimeters. For the age and gonad analyses in our study, we used 112 males and 115 females.

Lower SL	Upper SL	Males	Females	Proportion male	Proportion female
70	80	3	0	1.00	0.00
80	90	7	1	0.88	0.13
90	100	9	6	0.60	0.40
100	110	7	8	0.47	0.53
110	120	13	5	0.72	0.28
120	130	16	2	0.89	0.11
130	140	20	1	0.95	0.05
140	150	20	2	0.91	0.09
150	160	16	3	0.84	0.16
160	170	1	11	0.08	0.92
170	180	0	19	0.00	1.00
180	190	0	19	0.00	1.00
190	200	0	16	0.00	1.00
200	210	0	18	0.00	1.00
210	220	0	4	0.00	1.00

whelk. Males dominated the size range of 110–160 mm SL and ages of 7–9 years, and females dominated the size intervals above 160 mm SL and ages of 10–14 years (Tables 1 and 2). For the 137 dissected male channeled whelk, the maximum SL recorded was 175.0 mm and the maximum Wt was 490.6 g. Females typically grew larger than males, reaching a maximum SL of 214.2 mm and a maximum Wt of 930.0 g (n=155).

Penis length increased with SL in males (Table 3) The single exception was a 199.3-mm male that was removed from the study because histology showed that this whelk's gonad was nonfunctional. Its gonad was empty of any gonadal precursors and contained only connective tissue. The sex of the whelk could not be determined on the basis of the gonad. It was recorded as a male only because a small penis (15 mm in length) was detected; a mature male at 165 mm SL normally would have a penis approximately 37 mm long.

There was an exponential relationship between SL and Wt and a linear relationship between SL and LW. Covariance analysis of $\log_{10} Wt$ to $\log_{10} SL$ and *sex* (Table 4) showed that the male intercept and male slope were not significantly different from the female intercept and female slope. The results of this covariance analysis of $\log_{10} Wt$ indicate that $\log_{10} Wt$ regression equations for males and females are similar (Table 3). Because sex was not a significant factor, a combined regression equation that included both sexes was calculated and indicated that $\log_{10} Wt$ was significantly related to $\log_{10} SL$ (Tables 3 and 4). Covariance analysis of *LW* versus *SL* and *sex* showed that slopes differed between sexes and that male and female intercepts were similar (Tables 3 and 4). The LW covariance analysis indicates that males and females have a similar initial size, but male LW may differ from female LW as SL increases.

Growth rate

The von Bertalanffy growth models for the sexes were significantly different (Table 5; Fig. 4, A and B). The oldest female was 14 years old, and males reached a maximum age of 12 years. Males and females had similar SL until about the age of 4 years, after which the curve for males started to plateau (Fig. 4C). Females reached a larger maximum size and had higher annual growth rates than males (Fig. 4, C and D). The growth rates for males and females decreased as age increased (Fig. 4D). Covariance analysis of sqrt (*SL growth*) versus sqrt (*age*) and *sex* indicated that the intercepts were not significantly different, and the male slope was significantly less than the female slope (Table 4). The sqrt (*SL growth*) covariance analysis indicates that males and females had a similar initial size, but male and female growth rates differed over time.

Size at 50% maturity

Females classified in stage I of gonad development did not exceed 130 mm SL (Fig. 5A) and 8 years of age (Fig. 5C). Females identified as ED did not exceed 160

Table 2

Number and proportion of each sex by age in years for channeled whelk (*Busycotypus canaliculatus*) collected in August 2010 and July 2011 in Buzzards Bay, Massachusetts. For the age and gonad analyses in our study, we used 112 males and 115 females.

Age	Male	Female	Proportion male	Proportion female
4	1	1	0.50	0.50
5	5	5	0.50	0.50
6	12	10	0.55	0.45
7	27	5	0.84	0.16
8	26	5	0.84	0.16
9	27	8	0.77	0.23
10	9	21	0.30	0.70
11	3	34	0.08	0.92
12	2	15	0.12	0.88
13	0	5	0.00	1.00
14	0	6	0.00	1.00

Table 3

Regression equations, sample sizes, coefficients of multiple determination (R^2), standard errors, and *P*-values for penis length, whole wet weight (Wt), lip width (LW), and shell width (SW) as functions of shell length (SL), LW, or SW from our study of channeled whelk (*Busycotypus canaliculatus*) collected in August 2010 and July 2011 in Buzzards Bay, Massachusetts. Standard error (SE) is ±1 SE.

Regression	Equation	Sample size (n)	R^2	Standard error	*P*-value
Penis length	$-249.171 + 128.982 * \log_{10} SL$	92	0.801	4.048	<2.2e–16
Female $\log_{10}$Wt	$-4.437 + 3.139 * \log_{10} SL$	115	0.978	0.049	<2.2e–16
Male $\log_{10}$Wt	$-4.204 + 3.027 * \log_{10} SL$	112	0.956	0.053	<2.2e–16
Log_{10}Wt	$-4.385 + 3.114 * \log_{10} SL$	227	0.979	0.051	<2.2e–16
Female LW	$3.699 + 0.504 * SL$	115	0.976	2.779	<2.2e–16
Male LW	$6.453 + 0.473 * SL$	112	0.958	2.177	<2.2e–16
SW	$-9.607 + 1.233 * LW$	166	0.938	2.933	<2.2e–16
LW	$11.954 + 0.761 * SW$	166	0.938	2.304	<2.2e–16

mm SL (Fig. 5A) and 9 years of age (Fig. 5C). No LD or R females exceeded 190 mm SL (Fig. 5A), although there were females assigned as LD at the age of 12 years and females identified as stage R at the age of 14 years (Fig. 5C). The largest males classified as stage I were 120 mm SL; males identified as ED did not exceed 130 mm SL, and LD males did not exceed 140 mm SL (Fig. 5B). Similar to females in stages I and ED, males in stages I and ED did not exceed the ages of 8 years and 9 years, respectively; however, the ages of LD males also were not older than 9 years (Fig. 5D). Females were assigned to stage M as small as 159 mm SL and at 8 years of age, and males were classified in stage M as small as 104 mm SL and at 6 years of age (Fig. 5). Both males and females included relatively few LD individuals, indicating that spawning had not yet occurred and development was not yet complete.

Table 4

Covariance analysis on regressions of channeled whelk (*Busycotypus canaliculatus*) growth in weight (Wt), lip width (LW), and square-root (Sqrt) shell length (SL) from our study of this species collected in August 2010 and July 2011 in Buzzards Bay, Massachusetts. Sample size for each regression was 112 male and 115 female channeled whelk. The female intercept and slope are as stated. The actual male intercept is calculated by adding the male intercept estimate to the female intercept estimate. The actual male slope is calculated by adding the male slope estimate to the female slope estimate. Standard error (SE) is ±1 SE.

Regression	Coefficient	Estimate	Standard error	*P*-value
$\text{Log}_{10} Wt \sim \log_{10} SL * sex$	Female intercept	–4.437	0.103	<2e–16
	Female slope	3.139	0.046	<2e–16
	Male intercept	0.233	0.162	0.151
	Male slope	–0.112	0.075	0.138
$\text{Log}_{10} Wt \sim \log_{10} SL$	Intercept	–4.385	0.066	<2e–16
	Slope	3.114	0.030	<2e–16
$LW \sim \text{SL} * sex$	Female intercept	3.699	1.168	0.002
	Female slope	0.504	0.007	<2e–16
	Male intercept	2.754	1.814	0.130
	Male slope	–0.031	0.013	0.015
Sqrt ($SL\ growth$) ~ sqrt (age) * sex	Female intercept	7.919	0.052	<2e–16
	Female slope	–1.496	0.018	<2e–16
	Male intercept	–0.039	0.073	0.594
	Male slope	–0.209	0.026	4.63e–08

The average proportion of mature channeled whelk in each interval (10 mm SL or 5 mm LW) were plotted along with the fitted logistic maturity curves from the GLM for each sex in Figures 6A and 6B, respectively. Estimated male SM_{50} was 115.5 mm SL (SE 2.2), and 61.3 mm LW (SE 0.9) (Table 6; Fig. 6, A and B). Estimated female SM_{50} was 155.3 mm SL (SE 3.0) and 80.6 mm LW (SE 1.9) (Table 6; Fig. 6, A and B). Estimated male and female age at SM_{50} was 6.9 years (SE 0.2) and 8.6 years (SE 0.3), respectively (Table 6). Conversion of LW to SW through regression yielded an SM_{50} of 66.0 mm SW (SE 2.9) and 89.7 mm SW (SE 2.9) for males and females, respectively (Table 3). The SW regression was not sex specific. The current minimum size limit (69.9 mm SW) was equivalent to a size of 65.1 mm LW (SE 2.3) (Table 3). From the von Bertalanffy growth model, age at minimum size limit was calculated as 7.5 years (7.0–7.9 years) and 6.3 years (6.1–6.6 years) for males and females, respectively (Table 5).

Table 5

Estimates for the parameters of shell length (SL) or lip width (LW) for male and female channeled whelk (*Busycotypus canaliculatus*) from the von Bertalanffy growth model used in our study of this species in Buzzards Bay, Massachusetts, in August 2010 and July 2011. Each estimate is given as value ±1 standard error (SE). L_{inf}=theoretical maximum SL; K=growth coefficient; t_0=theoretical age at length 0.

Model	Sample size (n)	L_{inf}	K	t_0
Female SL	115	247.15 (21.63)	0.15 (0.04)	1.78 (0.59)
Female LW	115	126.40 (10.10)	0.16 (0.04)	1.73 (0.59)
Male SL	112	177.80 (20.01)	0.20 (0.08)	1.63 (0.88)
Male LW	112	89.11 (8.79)	0.22 (0.08)	1.40 (0.95)

Discussion

This article describes the first published study on reproductive maturity and growth in channeled whelk, and it provides the first estimates of size at maturity for this species. Males reached SM_{50} 2 years before females did. In addition, on the basis of SL, male SM_{50} was 40 mm less than female SM_{50}. Male channeled whelk reached SM_{50} at 66.0 mm SW (SE 2.9), which is below the minimum legal size limit in Massachusetts. However, females reached SM_{50} at 89.7 mm SW (SE 2.9), 20 mm greater than the minimum size limit. This result indicates that males have a greater chance of copulation before they are harvested than do females, many of which are captured before they reach sexual maturity. Females enter the fishery at an age of 6.3 years, approximately 2 years before they reach SM_{50}. In contrast, males enter the fishery at an age of 7.5 years, a few months after they reach SM_{50}. Although the annual growth rate was higher for female channeled whelk than for male whelk, females took longer to reach SM_{50}. Male waved

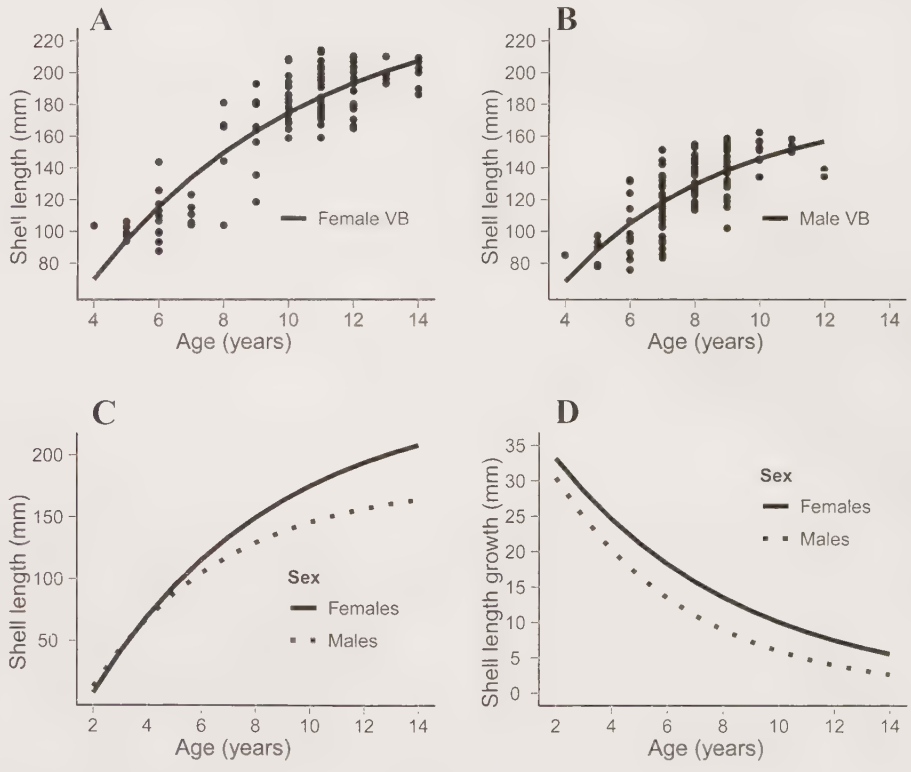

Figure 4

Results from the von Bertalanffy (VB) growth model used in our study of channeled whelk (*Busycotypus canaliculatus*) collected in Buzzards Bay, Massachusetts, in August 2010 and July 2011: (**A**) VB growth model of females (n=115); (**B**) VB growth model of males (n=112); (**C**) a comparison of male and female VB growth models; and (**D**) the calculated growth per year for males and females from the VB growth model.

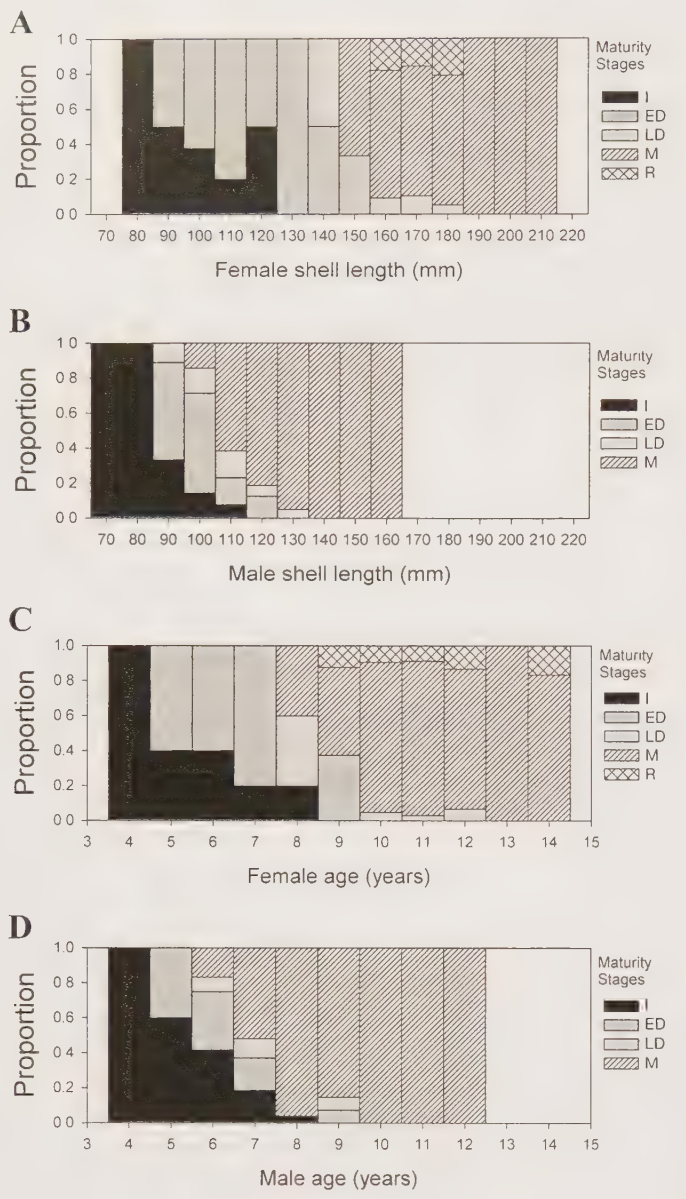

Figure 5

Proportions of channeled whelk (*Busycotypus canaliculatus*) classified at different stages of gonad development in our study of this species in Buzzards Bay, Massachusetts, in August 2010 and July 2011, shown by varying shell lengths (for [**A**] females and [**B**] males) and by varying ages (for [**C**] females and [**D**] males). The maturity stages used in our study and presented here are immature (I), early developing (ED), late developing (LD), mature (M), and recovering (R). For gonad analysis, we used 115 females and 112 males.

whelk also reach 50% maturity before females do, at an age of 3 years versus 4 years (Heude-Bertherlin et al., 2011). Sustainability of the channeled whelk population is of concern for this fishery because effort and incentives are biased toward catch of larger whelk, which are almost entirely female.

The highest growth rate for males and females occurred in the first few years of life. Growth rate progressively decreased as channeled whelk aged (Fig. 4D). There was a wide range of SL at each age for this species (Fig. 4, A and B). This variation also was evident in knobbed whelk in Delaware Bay (Bruce et al.[3]) and *Buccinum isaotakii* (Ilano et al., 2004), both of which were aged by examining opercula.

Harding (2011) reported an average size of 3.8 mm SL at hatching for channeled whelk cultured from hatch in the laboratory. At 171 days after hatching, the average SL was 48.4 mm; a linear growth model for age-at-length resulted in a growth rate of 0.21 mm/day (Harding, 2011). In our study of wild channeled whelk, the von Bertalanffy growth model predicted that an average SL of 48.4 mm would not be achieved until the age of 3 years, indicating a much slower growth rate for whelks in our study than the one found by Harding (2011). Although we did not capture channeled whelk younger than 4 years old, the von Bertalanffy growth model predicted that 3-year-old males and females would be only 41.3 mm SL and 43.5 mm SL, respectively (Fig. 4C; Table 5). The discrepancy in growth rates between channeled whelk in our study and the whelk in the Harding (2011) study possibly reflects individual variation, although food availability, predator abundance, and habitat may affect shell growth as well.

Channeled whelk held in laboratory tanks may be more protected from shell damage than channeled whelk in their natural environment. Channeled whelk have thin shells and can easily chip their shells (or siphons) while they feed or move around on the ocean floor. In addition, channeled whelk in a laboratory may be exposed to more food than they would be if they were in the ocean. Bourdeau (2010) reported that the frilled dogwinkle (*Nucella lamellosa*), a marine snail, had thicker shells and reduced shell growth when in the presence of red rock crab (*Cancer productus*). Food-limited snails did not significantly differ from snails exposed to crab, indicating food consumption, instead of a physiological response from predation, ultimately affected growth (Bourdeau, 2010). These factors that affect SL growth may partially explain the quicker growth rates in Harding (2011) compared with the rates found in our study.

Although knobbed whelk are in a different genus and have thicker shells than channeled whelk (Magalhaes, 1948), both whelks share similar growth pat-

terns. From the von Bertalanffy growth model, estimates of SL for 10-year-old male and female channeled whelk were 145.7 mm and 174.8 mm, respectively (Fig. 4C; Table 5). Kraeuter et al. (1989) reported knobbed whelk in the seaside lagoons of Virginia with SL of 176 mm after 10 years (average for ages 9–11). Bruce et al.[3] reported 10-year-old knobbed whelk in Delaware Bay with average SL of 112.8 mm and 127.6 mm for males and females, respectively. The Delaware Bay population of knobbed whelk appears stunted (possibly because of heavy fishing pressure from the whelk dredge fishery) when compared with the Virginia population. Error in aging opercula also could attribute to shorter SL at age in Delaware Bay. Channeled whelk and knobbed whelk share sexual dimorphism in maximum size. The largest male knobbed whelk in Delaware Bay reached an SL of 161 mm; whereas the largest female reached an SL of 197 mm (Bruce et al.[3]). In Buzzards Bay, Massachusetts, the second-largest male had an SL of 175 mm, but the largest female reached an SL of 214.2 mm. The largest male found in Buzzards Bay was deemed reproductively unviable and subsequently discarded from our analysis.

Table 6

Estimates of size at 50% maturity (SM_{50}) by shell length (SL), lip width (LW), and age for male and female channeled whelk (*Busycotypus canaliculatus*) collected for our study in Buzzards Bay, Massachusetts, in August 2010 and July 2011. We resampled each data set with replacement 1000 times with a bootstrap routine to calculate the resampled SM_{50} estimate and the bias and standard error (SE) of the original SM_{50} estimate. Bias is the difference of the original SM_{50} estimate from the resampled SM_{50} estimate. Standard error (SE) is ±1 SE around the original SM_{50} estimate.

Sex	Sample size (n)	Subject	SM_{50} estimate	Bias	SE
Female	115	Age	8.6 years	0.0014	0.3
		SL	155.3 mm	0.24	3.0
		LW	80.6 mm	0.11	1.9
Male	112	Age	6.9 years	–0.0027	0.2
		SL	115.5 mm	0.12	2.2
		LW	61.3 mm	0.04	0.9

Females had a slightly larger LW at larger SL when compared with males. This finding could be due to the presence of the nidamental gland, which takes up a large portion of shell volume in mature females. Female waved whelk invest more energy in reproduction than do males of that species: 3.84 $kj \cdot m^{-2} \cdot yr^{-1}$ versus 0.26 $kj \cdot m^{-2} \cdot yr^{-1}$, respectively (Kideys et al., 1993). The nidamental gland is essential for forming egg cases and may require channeled whelk females to spend more energy on reproduction than do males. Channeled whelk males only need to form a penis and testis, which may explain why males have a lower maximum size.

Males and females had a similar relationship of Wt to SL, although females grew to larger sizes. We expected that the weights of males and females would differ because females form ovaries and a nidamental gland, which would alter the relationship between Wt and SL at larger SL. Castagna and Kraeuter (1994) reported the seasonal gonad index of nidamental gland per meat weight (N/MW) at a range of 9.5–18.3% for female knobbed whelk. N/MW was lowest in the spring and in October; there has been no field observations reported for knobbed whelk spawning in the spring, although observed egg laying peaked in October (Castagna and Kraeuter, 1994). Despite the in-

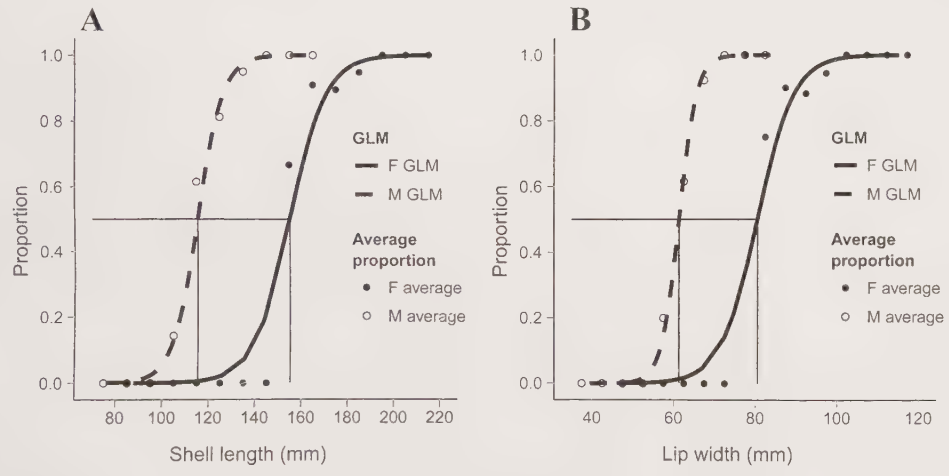

Figure 6

Size at 50% maturity (SM_{50}), on the basis of (**A**) shell length (SL) or (**B**) lip width (LW), for male (M) and female (F) channeled whelk (*Busycotypus canaliculatus*) collected in August 2010 and July 2011 in Buzzards Bay, Massachusetts. Lines indicate predictions from a generalized linear model (GLM); points represent the average proportion within each size interval of 10 mm SL or 5 mm LW. Vertical lines indicate SM_{50}. For age and gonad analysis, we used 115 females and 112 males.

crease in LW and the subsequent increase in volume of female shells, the lack of any observed difference in the relationship of Wt and SL could have occurred because the nidamental gland has a lower density than the density of muscle tissue.

During dissections of channeled whelk in our study, there were no signs of hermaphroditism; all whelk had either a penis or a nidamental gland. No whelk were found to contain both male and female gonads in histological sections. Male penis length increased with SL, a finding that also indicates channeled whelk may not be protandric hermaphrodites. In knobbed whelk, the penis of males that changed sex to females shrank to a round protuberance (Castagna and Kraeuter, 1994). The results of Castagna and Kraeuter (1994) indicate knobbed whelk can be protandric hermaphrodites, although sex is determined at birth and there is a 1:1 sex ratio at hatching (Avise et al., 2004).

We found an equal sex ratio for male and female channeled whelk at early ages, but later, males dominated at ages of 7–9 years and females dominated at ages of 10–14 years (Table 2). Males did not live as long as females (Table 2), and there was a greater proportion of males at the size range of 120–160 mm SL (Table 1). Both factors could explain why there were more males than females at the ages of 7–9 years. It also is possible that the fishery has inflated the proportion of males at smaller sizes. Males have a lower maximum size and mature at smaller sizes than females. We define large whelk as those individuals >160 mm SL because this size is slightly above the female SM_{50} and is equivalent to the 97.5 percentile of male SL (on the basis of a sample of 9460 whelk; B. Stevens, unpubl. data). Because the fishery is directed at catch of large whelk, which are mostly females, fishermen do not actively target males and males may accumulate at smaller sizes.

However, it is possible that knobbed whelk may exhibit protandric behavior in extreme conditions; Castagna and Kraeuter (1994) held only male knobbed whelk in the laboratory. Whelks generally grow slowly and move slowly, and, at low population levels, interaction between sexes may be minimal. Protandry in knobbed whelk may be opportunistic, and not every individual may be capable of it. In addition, unknown pollutants potentially could cause the sex change seen in knobbed whelk by Castagna and Kraeuter (1994). Whether or not protandry occurs in channeled whelk, the fishery will remain in peril if mature females are not protected.

There is a lack of evidence to support the hypothesis that protandry may occur in channeled whelk. Further, no channeled whelk with evidence of imposex were found in our study. In gastropods, tributyltin (TBT) causes higher testosterone, which in turn can cause the penis in male bruised nassa (*Nassarius vibex*) to develop earlier and males to mature earlier (Demaintenon, 2001). Female bruised nassa with imposex developed a penis, and that development caused sterility in some cases because the penis blocked the oviduct (Demaintenon, 2001). Females with imposex can be confused with sequential hermaphrodites in sex transition because individuals may have both male and female sex organs. Castagna and Kraeuter (1994) indicated that knobbed whelk could be sequential hermaphrodites because some male knobbed whelk switched sex to female in the laboratory, and some of the newly formed female knobbed whelk laid viable egg strings. However, it is possible that their knobbed whelk were originally females with imposex, or that sex change was the result of social and physiological effects of confinement in a laboratory setting.

Gonad histology of the "male" channeled whelk that was abnormally large (more than 20 mm longer than the second-largest male) revealed that its gonad was nonfunctional. The gonad was empty of any male or female gonadal precursors and contained only connective tissue. The sizes of the penis and gonads were much smaller in this abnormally large male than in fully mature male channeled whelk in the size range of 150–160 mm SL. Because the testis and penis were very small, this whelk may never have been reproductively viable or was too old to reproduce. However, it is still possible that this large male was exhibiting protandric or even imposex conditions.

The gonads of only 3 of 115 female channeled whelk contained different dominant stages in the replicate slides; males did not have different dominant stages (n=112). This finding indicates that gonadal development was mostly synchronous. Mann et al. (2006) reported asynchrony for 2 of 3 male specimens of *Rapana venosa* but not for female specimens collected in June from the Chesapeake Bay. The gonad developmental stages determined for channeled whelk in this study are point-in-time estimates and do not provide a seasonal evaluation. Gonad samples from our study were collected in July and August, most likely at the beginning of the spawning season for channeled whelk (Betzer and Pilson, 1974). The I and ED stages were differentiated to determine when spermatozoa (males) and nuclei (females) were first produced.

Male maturity was determined mostly by the presence and amount of spermatozoa. We believed that a male was capable of spawning effectively if it contained spermatozoa in at least 50% of its tubules, and we labeled such whelk in our study as mature. A LD-stage male that contained spermatozoa in 25–50% of its tubules could potentially spawn, but it may not contribute enough spermatozoa to be considered effective.

There is no paternity information on egg strings of channeled whelk, but this species could follow a similar reproductive strategy to that of knobbed whelk. Walker et al. (2005) reported sex-linked markers in knobbed whelk that can be used to determine the biological parents of embryos. A knobbed whelk egg string contained 7 different fathers, indicating that the female knobbed whelk most likely used a "well-blended sperm pool" for fertilization (Walker et al., 2007). Female knobbed

whelk may copulate with multiple males to increase fitness or because males do not excrete enough spermatozoa to fertilize an entire female brood. If male channeled whelk copulate with multiple females, then it is possible that males do not use all their spermatozoa on one female; this "sperm conservation" strategy of sperm competition is common among snow crab (*Chionoecetes opilio*) when the ratio of females to males is high (Rondeau and Sainte-Marie, 2001; Sainte-Marie et al., 2002).

Female maturity was determined mainly by the presence of VOs. We assumed a gonad with VOs that covered more than half the cross section could be capable of reproduction, although fecundity could be lower in a female with such a gonad than in a fully matured female. A LD-stage female contained VOs in less than half the cross section and most likely would not reproduce until 1–2 years later.

Staging of gonad development for male channeled whelk was more consistent than staging for females because there were a greater proportion of males at smaller SLs. Males accumulated at smaller size classes (110–160 mm SL) because of a slower growth rate and a lower maximum size than the growth and size of females. In contrast, females grew rapidly past the shorter SL of males. Male and female channeled whelk classified as stage LD may not spawn until the following year because channeled whelk typically grow slowly and would have to overcome a large deficit in gonadal development in just a few months to be considered mature. This large deficit in gonadal development is especially evident in females that probably invest more energy into reproduction than males. Females in stage R were not present at sizes >190 mm SL (Fig. 5A).

It is not known if larger females (>190 mm SL) spawn every year, every other year, or multiple times per year. Bruce et al.[3] suggested that female knobbed whelk with gravid ovaries (comparable to a mature female) may stay in this state year-round and consequently, not spawn annually. In our study, smaller female channeled whelk (<190 mm SL) classified in the R stage could have spawned earlier in the year or in the previous year. Large females also may not completely deplete their ovaries when they spawn, as we observed in some smaller females, and, therefore, may have a shortened generation time. Large females also may have been ready to spawn after we sampled them in July and August. This circumstance may have been the reason why few females were classified as stage R. Reduced fecundity at older age or larger size, although unlikely, also could be a reason that no females in stage R were found at sizes >190 mm SL.

Conclusions

On the basis of the morphological and histological evidence collected in our study, we suggest that most channeled whelk do not change sex, although channeled whelk under some conditions may exhibit protandric-like symptoms (as did the unusually large male in our study). We did not find any channeled whelk with both a penis and nidamental gland or both ovary and testes. All female whelk contained a nidamental gland and ovary. All male whelk contained a penis and testes, except for the large male that contained a penis and an inactive gonad. Penis length increased with SL in males, indicating that channeled whelk are not protandric. Females reached a larger maximum size and age and had a quicker growth rate than males. With the current minimum size limit, this fishery captures small females before they reach SM_{50} and males just after SM_{50}. To prevent the occurrence of overfishing, fishery managers need to consider the sex-specific growth rates, SM_{50}, and fecundity of channeled whelk. More information on population estimates are needed to understand if the channeled whelk population in Massachusetts is being overfished. The results from this study provide information necessary for managers to work with lawmakers to enact appropriate legislation on size limits to secure the longevity of the Massachusetts whelk fishery and to allow potential mature females to spawn.

Acknowledgments

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Mortality and movement of Yellowtail Flounder (*Limanda ferruginea*) tagged off New England

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Abstract—From 2003 to 2006, 44,882 Yellowtail Flounder (*Limanda ferruginea*) were captured and released with conventional disc tags in the western North Atlantic as part of a cooperative Yellowtail Flounder tagging study. From these releases, 3767 of the tags were recovered. The primary objectives of this tagging program were to evaluate the mortality and large-scale movement of Yellowtail Flounder among 3 stock areas in New England. To explore mortality, survival and recovery rate were estimated from traditional Brownie tag-recovery models fitted to the data with Program MARK. Models were examined with time- and sex-dependent parameters over several temporal scales. The models with a monthly scale for both survival and recovery rate had the best overall fit and returned parameter estimates that were biologically reasonable. Estimates of survival from the tag-recovery models confirm the general magnitude of total mortality derived from age-based stock assessments but indicate that survival was greater for females than for males. In addition to calculating mortality estimates, we examined the pattern of release and recapture locations and revealed frequent movements within stock areas and less frequent movement among stock areas. The collaboration of fishermen and scientists for this study successfully resulted in independent confirmation of previously documented patterns of movement and mortality rates from conventional age-based analyses.

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The Yellowtail Flounder (*Limanda ferruginea*) is one of the principal resources of the groundfish complex in the northeastern United States, with major fishing grounds on Georges Bank, off southern New England, and off Cape Cod. The fishery for Yellowtail Flounder is among the most productive and valuable in New England, yielding 1832.5 metric tons and $4.78 million to U.S. fishermen in 2011 (NMFS, 2012). However, the potential yield of Yellowtail Flounder is much greater than the current yield. The estimated maximum sustainable yield from the 3 stocks in New England is 29,483.5 metric tons (NEFSC[1]). The 3 stock areas examined in this study were Cape Cod–Gulf of Maine, Georges Bank, and southern New England–Mid-Atlantic.

Despite substantial investments in sampling Yellowtail Flounder fisheries and resources, uncertainty persists in the age-based stock assessments from the 2008 Groundfish Assessment Review Meeting of Northeast Fisheries Science Center (NEFSC[1]). The sources of this uncertainty are not well understood but may result from the movement of flounder among stock areas, lack of information on the effect of closed areas on population dynamics, insufficient surveying of areas closed to fishing, inaccurate age determinations, misrepresentative sampling of distributional patterns, underreported catch, or inaccurate assumptions about natural mortality (NEFSC[1]). In 2002, a cooperative tagging study was designed to provide independent information on mortality and movement, to complement the current stock assessment methods for Yellowtail Flounder, and to improve the reliability of scientific advice for effective fishery management (NEFSC[2]).

Movement of Yellowtail Flounder off New England has been addressed

[1] NEFSC (Northeast Fisheries Science Center). 2008. Assessment of 19 northeast groundfish stocks through 2007: report of the 3rd Groundfish Assessment Review Meeting (GARM III), Northeast Fisheries Science Center, Woods Hole, MA, 4–8 August 2008. NMFS, Northeast Fish. Sci. Cent. Ref. Doc. 08-15, 884 p. + xvii. [Available from National Marine Fisheries Service, 166 Water Street, Woods Hole, MA 02543-1026.]

[2] NEFSC (Northeast Fisheries Science Center), Workshop Organizing Committee (S. Tallack, ed., P. Rago, chairperson, T. Brawn, workshop coordinator, and (alphabetically) S. Cadrin, J. Hoey, and L. Taylor Singer). 2005. Proceedings of a workshop to review and evaluate the design and utility of fish mark-recapture projects in the northeastern United States; Nonantum Resort, Kennebunkport, Maine, 19–21 October 2004. U.S. Dep. Commer., Northeast Fish. Sci. Cent. Ref. Doc. 05-02; 141 p. [Available from National Marine Fisheries Service, 166 Water St., Woods Hole, MA 02543-1026.]

by several historical and more recent tagging studies. A study that examined fish tagged and released off the northeastern coast of the United States from 1942 to 1949 concluded that groups were relatively localized and exhibited short seasonal migrations. Most tagged fish were recovered within 80 km of the release site and little mixing was observed between fishing grounds, except for frequent movement from the Mid-Atlantic Bight to the waters of southern New England (Royce et al., 1959). Lux (1963) confirmed these observations and concluded that groups of Yellowtail Flounder move seasonally within fishing grounds, and a small amount of seasonal mixing occurs among groups. More recent tagging studies of Yellowtail Flounder in Canadian waters have confirmed the sedentary nature of this species. Fish tagged from 3 studies on the Grand Bank, Canada, traveled an average of 59 km (Walsh and Morgan, 2004). A recent summary of all previously published studies that detailed Yellowtail Flounder movements off the northeastern United States indicated 95% residence in current stock areas (Cadrin, 2010).

The cooperative Yellowtail Flounder tagging study described in this article was designed to address sources of uncertainty in Yellowtail Flounder stock assessments. Two objectives of this study were to provide 1) estimates of mortality independent of mortality estimates from current Yellowtail Flounder stock assessments and 2) extensive release and recovery information and documentation of movement of fish between stock areas.

Materials and methods

The general approach to tagging in this study involved a sampling design with geographic coverage that represented the entire Yellowtail Flounder resource off New England. All phases of the proposed research, from the field protocol to public outreach, were developed cooperatively with New England groundfish fishermen. We contracted commercial fishermen and their vessels to work with scientists to tag and release fish in all stock areas. The geographic design was developed on the basis of fishing areas, with releases in each area proportional to values of relative abundance of Yellowtail Flounder from groundfish surveys conducted by the Northeast Fisheries Science Center. The field protocol and analytical design were considered during peer review to be valid approaches to meet the objectives of this tagging study (NEFSC[2]).

Fish were captured with commercial otter trawls or gillnets with large mesh (16.5 cm) and relatively short trawl tows (30 min) or gillnet sets (<6 h). Yellowtail Flounder were identified by using Collette and Klein-MacPhee (2002). All legal-size fish (>33 cm in fork length) in viable condition, and some sublegal-size fish from tows in low-density areas, in the southern New England–Mid-Atlantic stock area were tagged with Petersen disc tags (22-mm in diameter). Viability was classified as *excellent*, *good*, or *poor*. Excellent fish exhibited body flexion and operculum or mouth movement, and no apparent damage from capture. Good fish also exhibited activity but had minor damage (e.g., scale loss, minor abrasion, and net marks). Poor fish, which exhibited no activity and had major abrasions or bleeding, were not tagged. Fish were released during the spawning season (May–August), with the exception of 1% of the releases, which occurred in the autumn of 2003.

Tags were collected from fish recaptured from a year-round commercial fishery with some seasonal geographic closures. The reward system for reporting recaptures included a $1000 lottery for all returned tags and 280 high-value ($100) rewards. The outreach system involved reward posters, brochures, a website (http://www.cooperative-tagging.org), annual letters to Yellowtail Flounder fishermen, press releases, and a toll-free phone number. Patterns of tag-recovery rates were analyzed statistically with contingency tables (*G*-test; Sokal and Rohlf, 1995) of frequencies of releases and reported recaptures by sex, size, condition code, and damage code.

To analyze the Yellowtail Flounder tagging data, Program MARK was used to fit multiperiod tagging models (White and Burnham, 1999). Program MARK facilitates the application of various types of mark-recapture models and estimates model parameters through the use of maximum likelihood. Brownie-type dead-recovery models (Brownie et al., 1985) were used to estimate the probability of Yellowtail Flounder survival (S) and a recovery rate probability (f). Recovery rate in this study was a compound parameter that represented the probabilities that a tagged fish was captured, the tag was retrieved, and the tag was reported. The model assumes that the probabilities of survival and recovery are the same for all marked animals and that tagging is instantaneous during sampling occasions.

A preliminary analysis, in which alternative tagging models with time-steps over different temporal scales (weekly, monthly, biannual and annual) were examined, indicated that the data were best suited for models with a monthly time step. Recovery data were entered into Program MARK with a classic recovery matrix format (Brownie et al., 1985; White and Burnham, 1999). The recovery matrix was examined with a suite of models that exhibited both time-dependent and constant survival, as well as time-dependent and constant recovery rate. Sex was included in the model as a group effect on survival and recovery rate. In addition, commercial catch was used as a proxy for fishing effort and was explored as a covariate on recovery rate in multiple models. Matrices of expected values were developed for each model, and recoveries were modeled as multinomial random variables. Parameters were estimated with maximum likelihood estimation.

Akaike's information criterion (AIC) was used to rank and select the model that achieved an optimal

balance between the parsimony of the model and the goodness-of-fit, where parsimony decreases as the number of parameters in the model increases. Model fit was judged with the model likelihood (L):

$$\mathrm{AIC} = 2K - 2\ln(L),$$

where K = the number of parameters.

To determine whether the general model (fully parameterized) was a reasonable fit to the tag-recovery data, goodness-of-fit was tested. A simulation procedure was used to calculate an estimate of overdispersion ($\hat{c}$). Data were simulated at varying levels of overdispersion (simulated c), and the deviance of each data set was divided by the degrees of freedom to obtain a range of values. A logistic regression was used to estimate the level of $\hat{c}$ where 50% of the simulated values were above and 50% were below the observed deviance divided by degrees of freedom of the general model. A model solution that perfectly conforms to the assumed error distribution would produce an expected variance equal to the observed variance and would have a $\hat{c}$ value of 1.0. Deviations of $\hat{c}$ above or below 1.0 indicate over- or underdispersion, respectively. Generally, a $\hat{c}$ value >3.0 indicates poor model fit because the model deviance is greater than the expected deviance (Lebrenton et al., 1992; Burnham and Andersen, 2002).

To account for $\hat{c}$ and for differences in effective sample size (N), a quasi-likelihood adjusted AIC ($\mathrm{QAIC_c}$) was used to adjust fit of the top selected models (Anderson et al., 1994; Burnham and Anderson, 2002):

$$QAIC_c = 2K + \frac{-2\ln(L)}{\hat{c}} + \frac{2K(K+1)}{N-K-1}.$$

The adjusted results from the top ranked models determined through the model selection criterion were then examined for biologically realistic parameter estimates. Models that estimated multiple parameters at their boundaries (e.g., S=1.0) were rejected in favor of the next ranked model.

Results

Researchers worked with commercial fishermen to tag 44,882 Yellowtail Flounder with conventional disc tags, and 3767 of these tags were recovered from the commercial fishery. Of all the lottery tags and $100 high-reward tags, 8% and 14% were returned, respectively. The relative return rate of lottery tags to high-value tags indicates a 59% reporting rate, assuming that 100% of the high-reward tags were reported (Table 1). The results from the analysis of observed recovery rates by sex, size, condition code, and damage code indicate that females had a greater recapture rate than males (particularly small males). Fish categorized as good had the same recovery rates as fish that were excellent. All damage codes had similar recovery rates, except for the slightly lower recovery rates for those fish with net marks (5% recovered) and those showing evidence of lymphocystis (3% recovered).

Table 1

Total releases and recaptures of tagged Yellowtail Flounder (*Limanda ferruginea*) by tag type from 2003 to 2006 in a cooperative tagging study off New England. The ratio of recovery rate from lottery tags and high-value tags indicates a 59% reporting rate, assuming 100% reporting of high-value tags.

Tag type	Releases	Recovered	Percentage recovered
Lottery tags	44,501	3713	8.3
$100 tags	381	54	14.2
Total	44,882	3767	8.4

Releases occurred in monthly batches over a 39-month period from June 2003 to August 2006, mostly in summer (Fig. 1). The full recovery matrix (with known month of capture) included a total recapture rate of 7.9% (Table 2). There was a higher rate of recapture for females (8.4%) than for males (6.5%).

Mortality

The release and recovery data were audited, and only tags with both location and fish sex information were included in the modeling. The final recovery matrix used to estimate survival included 43,907 releases and 3457 recaptures. Several model variations with both time-dependent and time-independent parameter estimates and with sex-dependant parameter estimates were successfully fitted to the data.

All of the top-ranked models determined through model selection had a time-dependent recovery rate parameter (f_t) with varying levels of sex dependence on both recovery rate and survival (S_g). Results from procedures for the simulation of goodness of fit indicate that the general model fitted the tag-recovery data well, returning a $\hat{c}$ estimate of 2.12 (Fig. 2). After the goodness-of-fit adjustment to the models, full weight was given to the model with a time-dependent survival and time-dependent sex-specific recovery rate. The 2 best models had a time-dependent survival estimate. However, many of the estimates were at the upper boundary of survival (S=1.0) because of sparseness in the data, and therefore the 2 models with time-dependent survival were not considered. The optimum model was a model with constant survival and time- and sex-dependent recovery rate (Table 3). On the basis of goodness-of-fit and model validation diagnostics, the optimum model appears to be a reliable representation of the data.

A constant rate of survival of 0.89/month with a standard error of 0.016 was estimated from the best model. The annual rate of survival was calculated at

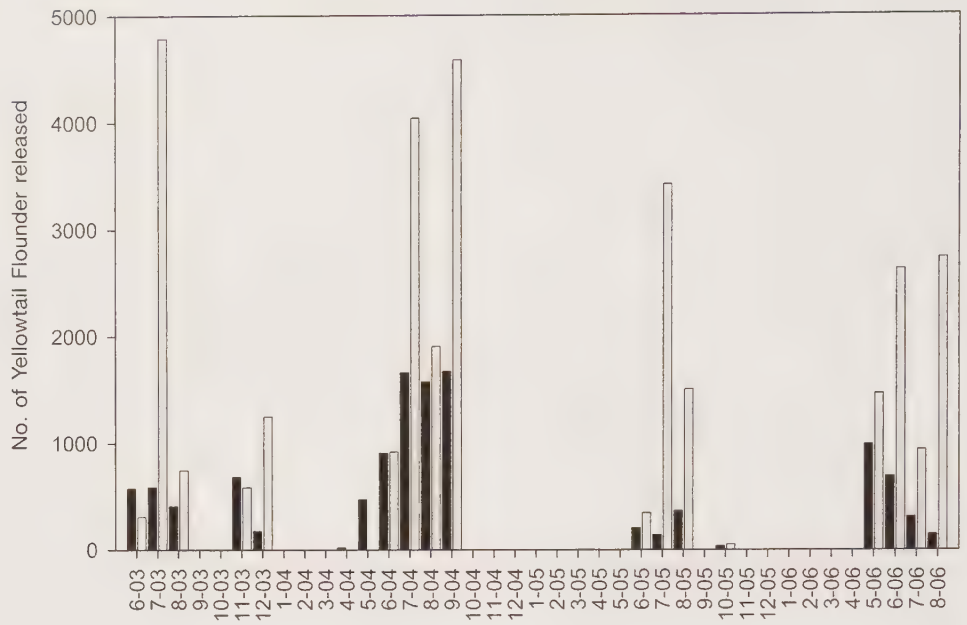

Figure 1

Number of tagged Yellowtail Flounder (*Limanda ferruginea*) released by month off New England from June 2003 to August 2006 for this tagging study. Black bars indicate males released, and light gray bars indicate females released.

0.25, a value equivalent to a total annual mortality (Z) of 1.4. Abundance-weighted F-values for age 6+ Yellowtail Flounder from an age-based stock assessment were 0.49, 1.49, 1.01, and 0.85, for the years from 2003 to 2006, respectively. The tag-based estimate of Z is within the range of these stock-assessment F-values, assuming a natural mortality of 0.2 for these years (Fig. 3). Estimates indicate that recovery rates ranged from 0.001 to 0.078 for males and 0.001 to 0.037 for females. Noticeable peaks in the recovery rate for females were observed in the period of May–August of each year (Fig. 4). These peaks were present but less evident in the trend for male recovery rate. Confidence intervals of recovery rate estimates indicate that recovery rates were greater in summer for females, but not for males, presumably because the estimates for females were more precise.

Movement

Results indicate frequent movements within the Cape Cod–Gulf of Maine and Georges Bank stock areas with less frequent movement among stock areas. Recapture data with known recapture locations indicate 96% residence in the Cape Cod–Gulf of Maine stock area (with 3% movement to the Georges Bank stock area and 1%

Table 2

Annual data of release and recapture of tagged Yellowtail Flounder (*Limanda ferruginea*) used to estimate survival in this study of the mortality and movement of this species off New England.

Year marked	Number marked	Number recaptured/year					Total recaptured	Percentage recaptured
		2003	2004	2005	2006	2007		
2003	10,122	526	403	72	18	3	1022	10.1
2004	17,783		628	492	96	18	1234	6.9
2005	6084			440	96	23	559	9.2
2006	9918				490	152	642	6.5
Totals	43,907	526	1049	1004	700	196	3457	7.9

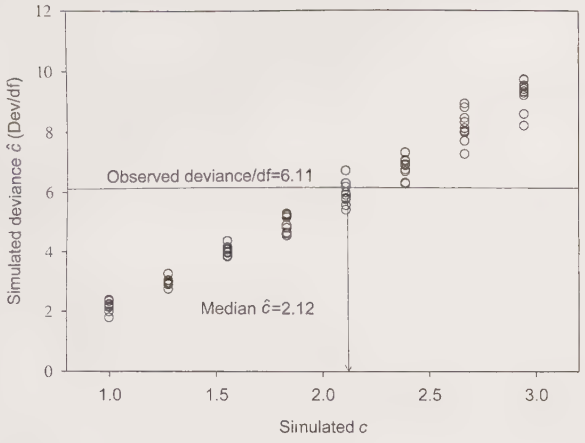

Figure 2

Results from a goodness-of-fit test used to estimate overdispersion ($\hat{c}$) for the general model fitted to tag-recovery data (time- and sex-dependent parameterization) for Yellowtail Flounder (*Limanda ferruginea*) tagged off New England from June 2003 to August 2006. The estimates of simulated deviance $\hat{c}$ are shown for a range of simulated c values. The value of $\hat{c}$, determined through logistic regression, is the point (6.11) where 50% of the simulated values fall above and 50% fall below the observed deviance $\hat{c}$ for the general model. df=degrees of freedom. Dev=deviance.

movement to the southern New England–Mid-Atlantic stock area), 98% residence on the Georges Bank stock area (with 1% movement to the Cape Cod–Gulf of Maine stock area and <1% movement to the southern New England–Mid-Atlantic), and 26% residence in the southern New England–Mid-Atlantic stock area (with 63% movement to the Georges Bank stock area and 10% movement to the southern Cape Cod–Gulf of Maine stock area; Table 4). However, most movement from southern New England was observed for Yellowtail Flounder released on Nantucket Shoals, near the boundary with adjacent stock areas (Fig. 5).

Discussion

The numbers of releases of tagged fish and tag recoveries in this study were 10 times greater than the numbers from any previous study of Yellowtail Flounder movement or mortality. The results from this study provide updated inferences of movement patterns and an independent estimate of mortality. Tag-recovery modeling and its application in fisheries research has increased in popularity over the past decade and has become an important tool to fisheries management (Pine et al., 2003). Large-scale tag-recapture studies provide insights into fish movement and population dynamics that are separate (except for their reliance on fishery recaptures) from the fishery-dependent and research survey data and methods used in conventional stock assessments. The results from tagging analyses should be of particular interest when there are suspected inconsistencies with the stock assessment data and analyses, as is the case for Yellowtail Flounder stocks off New England.

The results from tag-recovery modeling in this study are consistent with the perception that the Yellowtail Flounder resource in New England is experiencing an intense rate of mortality. The total annual mortality estimate of 1.4 derived from patterns of tag recovery is similar to stock-specific, age-based mortality estimates from the Yellowtail Flounder assessments for 2003 to 2006 (NEFSC[1]; Fig. 3). The results from this study demonstrate that models typically used in quantitative ecology (e.g., the Brownie tag-recovery model) can complement conventional methods for fisheries stock assessment. The advantage of the Brownie model is that survival estimates are not conditional on an assumed natural mortality rate, and recovery rates are a composite of exploitation rate (i.e., natural and fishing

Table 3

The top 5 most highly ranked models adjusted for a $\hat{c}$=2.12 that were fitted to tag-recovery data in this study of the mortality and movement of Yellowtail Flounder (*Limanda ferruginea*) tagged off New England from 2003 to 2006. Models were ranked by quasi-likelihood adjusted AIC ($QAIC_c$). Survival (S) and recovery rate (f) were estimated by month (t), for the entire time series (.), and by sex (g). The optimal model (in bold type) was chosen on the basis of rank and parameter estimates that were biologically reasonable.

Model	$QAIC_c$	Delta $QAIC_c$	$QAIC_c$ weight	Model likelihood	Number of Parameters	Qdeviance
S(t) f(g*t)	19765.56		Multiple boundary estimates for survival			
S(t) f(t)	19813.70		Multiple boundary estimates for survival			
S(.) f(g*t)	**19825.54**	**0.00**	**0.73**	**1.00**	**111**	**1181.05**
S(g) f(g*t)	19827.51	1.97	0.27	0.37	112	1181.01
S(g*t) f(t)	19847.22	21.68	0.00	0.00	163	1098.07

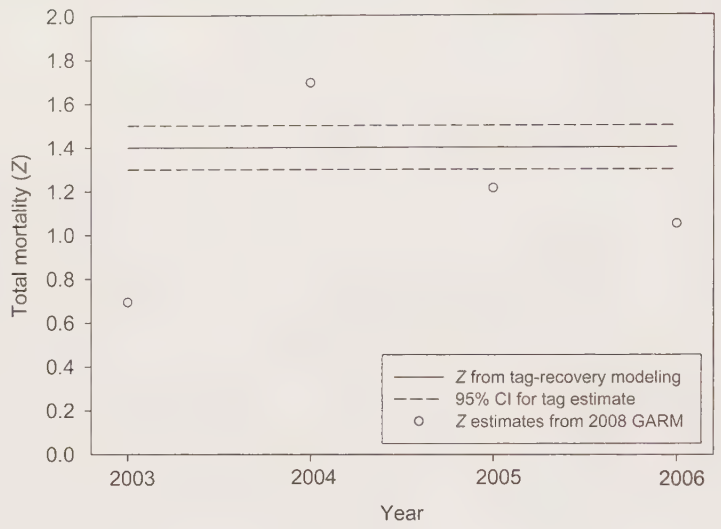

Figure 3

Estimates of total mortality (Z), with 95% confidence intervals (CIs), of Yellowtail Flounder (*Limanda ferruginea*) from 2003 to 2006 from tag-recovery modeling in this study and the stock assessments from the 2008 Groundfish Assessment Review Meeting (GARM) of the Northeast Fisheries Science Center.

mortality), survival from the tagging process, tag retention, and tag reporting (Brownie et al., 1985).

The mortality estimates derived from the tagging data corroborate stock assessment estimates; however, tag estimates could be inflated if a model assumption was violated. For the tagging models, all marked fish were assumed to have the same probability of survival and recapture by the fishery. This assumption is often violated when newly released fish do not fully mix with the population and have a different recapture probability (Hoenig et al., 1998). A close examination of the full residual matrix for Yellowtail Flounder did not reveal any patterns consistent with nonmixing, which is typically represented with a consistent residual along the recovery diagonal (Latour et al., 2001). Tagging-induced effects, both directly induced mortality and short-term tag loss, also could affect survival estimates. Experiments with tanks and cages designed to test the tag retention and tag-induced mortality of Yellowtail Flounder have indicated that these effects were not a concern (Alade, 2008). Any possible influence from these 2 effects likely would remain constant throughout the study and would not influence the survival estimates significantly.

Finally, the tag-recovery models used in this study did not estimate tag loss and tags were assumed not lost or missed. A fish that loses its tag is equivalent to a dead fish when it comes to the estimation of survival (Brownie et al., 1985). The Peterson disc tags used in this study are secure tags that pass through the body of a flounder and are anchored on both the dorsal and ventral surfaces. These tags have been widely applied in fish tagging studies and are known for a very high retention rate (Thorsteinsson, 2002). In addition, a long-term holding study with some fish held for more than 1 year showed 100% tag retention, and therefore tag loss was expected to be minimal (Alade, 2008).

Although the tag-recovery estimate of survival is consistent with results from age-based stock assessments, several aspects of this tagging study should be considered in future assessments. The difference in recovery rate between the sexes indicates that population dynamics may differ between males and females. The lower recovery rate of males could be a result of greater natural mortality—a finding that is consistent with sexually dimorphic growth rates and maximum sizes of Yellowtail Flounder (Lux and Nichy, 1969). However, sex-based recovery rates also could result from differences in catchability between sexes.

The possibility of substantial movement between stock areas (e.g., from southern New England to Georges Bank) may also influence population dynamics of Yellowtail Flounder (Hart and Cadrin, 2004). Analyses of simulated release and recapture data, with a data structure consistent to the data used in this study, indicate that movement and mortality cannot be simultaneously estimated because of highly correlated movement parameters (Alade, 2008). Therefore, simultaneous estimation of both movement and mortality may require an integrated analysis of data from tagging surveys, fisheries surveys, and resource surveys (Maunder, 2001; Goethel et al., 2010).

A summary of all published yellowtail flounder movements off the northeast United States, including those reported here, has revealed that juveniles and adults do not frequently move among fishing grounds (Cadrin, 2010). The movement from the southern New England–Mid-Atlantic stock area to the Georges Bank stock area observed in the study described in this article is greater than the movement reported in previous studies. However, most releases in the southern New England–Mid-Atlantic stock area were near the boundary because that location was the only one sampled in the stock area that had high densities of Yellowtail Flounder. The pattern of tag recoveries (Fig. 5) and previous analyses of movement trends from surveys (Cadrin, 2010) indicate that the southwestern portion of Georges Bank and Nantucket Shoals is an area of stock mixing, and that relative stock size may influence stock composition in that area. Although the study described here was not designed to estimate seasonal movement, the results of this research are consistent with those of previous tagging studies of Yellowtail Flounder off New

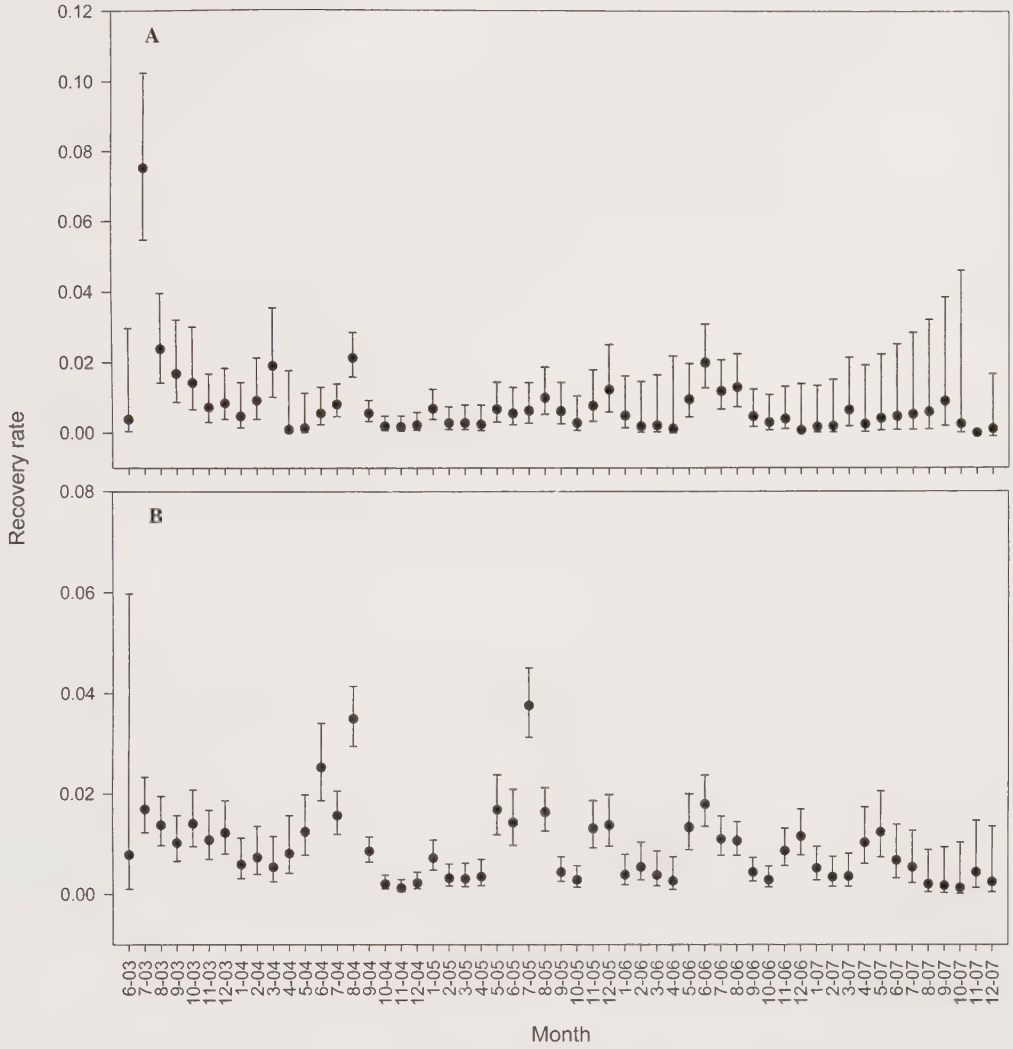

Figure 4

Recovery-rate probabilities by month for (**A**) male and (**B**) female Yellowtail Flounder (*Limanda ferruginea*) from the optimal model used in this study of the mortality and movement of this species from 2003 to 2006 off New England. The optimal model was the model with a constant survival and time- and sex-dependent recovery rate. Error bars represent 95% confidence intervals.

England that have indicated short, seasonal movement patterns (Royce et al., 1959; Lux, 1963).

Conclusions

Current stock assessments of Yellowtail Flounder provide valuable information for fishery management, although several major sources of uncertainty are present (NEFSC[1]). The results of this study address 2 important sources of uncertainty in the Yellowtail Flounder assessments: estimates of mortality and large-scale movement patterns among stock areas.

The results from the tag-recovery modeling in this study confirm that the Yellowtail Flounder population is experiencing a high level of mortality. These results were derived from data independent of the stock assessment data, and although we were able to confirm high levels of mortality, the direct cause remains unknown. In addition to modeling mortality, the pattern of tag-recovery locations from this study provides an updated look at stock mixing. Movement from the southern New England–Mid-Atlantic stock area to the Georges Bank stock area was greater than previously observed. However, some of this perceived movement was attributed to the study design and the locations

Table 4

Residence and movement of tagged Yellowtail Flounder (*Limanda ferruginea*) from 2003 to 2006 among managed stock areas off New England. Three stock areas were used in this study: Cape Cod–Gulf of Maine (CC-GOM), Georges Bank (GB), and southern New England–Mid-Atlantic (SNE-MA).

	Recapture area				Recapture percentage		
Release area	CC–GOM	GB	SNE–MA	Total	CC–GOM	GB	SNE–MA
CC–GOM	1049	38	10	1097	0.96	0.03	0.01
GB	32	2307	11	2350	0.01	0.98	0.01
SNE–MA	14	86	36	136	0.10	0.63	0.26
Total	1095	2431	57	3583			

where tagged fish were released. Tag-recovery patterns indicate that Yellowtail Flounder carried out frequent movements within stock areas and less frequent movement between areas. It is unlikely that uncertainties in the Yellowtail Flounder assessments are a result of substantial movement between stock areas.

This study provides insight into important uncertainties associated with the population dynamics of Yellowtail Flounder stocks off New England. The results from this study can be used to inform future assessments and provide additional information to aid in the management of this species.

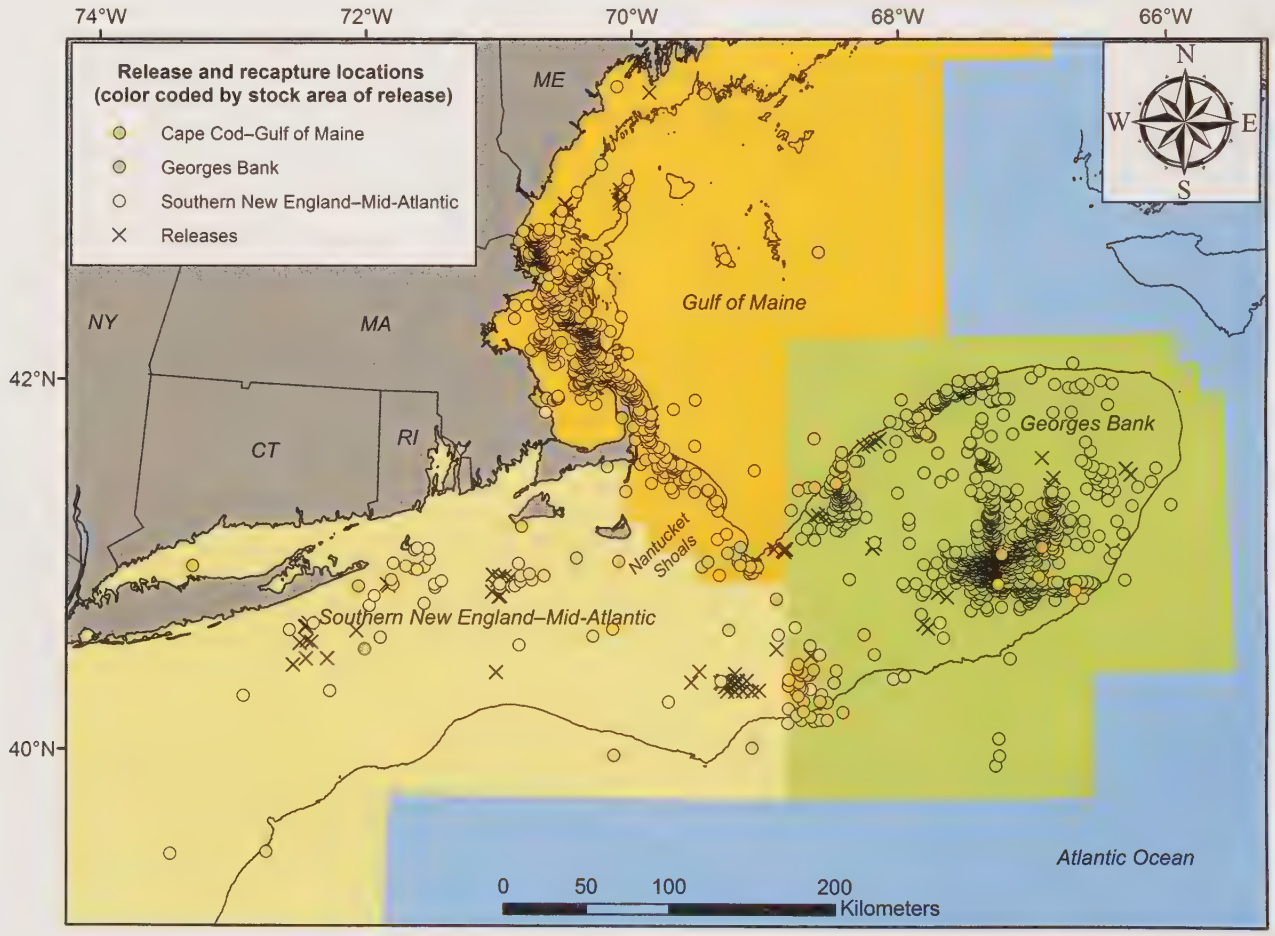

Figure 5

Map of the locations of release and recovery of tagged Yellowtail Flounder (*Limanda ferruginea*) in 3 stock areas off New England from 2003 to 2006. Recaptures are color-coded on the basis of the stock area where they were tagged and released. The 3 stock areas examined in this study were Cape Cod–Gulf of Maine (gold block), Georges Bank (green block), and southern New England–Mid-Atlantic (yellow block).

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For general style, follow the U.S. *Government Printing Office Style Manual* (2008) [available at http://www.gpoaccess.gov/stylemanual/index.html] and *Scientific Style and Format: the CSE Manual for Authors, Editors, and Publishers* (2006, 7th ed.) published by the Council of Science Editors. For scientific nomenclature, use the current edition of the American Fisheries Society's *Common and Scientific Names of Fishes from the United States, Canada, and Mexico* and its companion volumes (*Decapod Crustaceans, Mollusks, Cnidaria and Ctenophora*, and *World Fishes Important to North Americans*). For species not found in the above mentioned AFS publications and for more recent changes in nomenclature, use the Integrated Taxonomic Information System (ITIS) (available at http://itis.gov/), or, secondarily, the California Academy of Sciences *Catalog* of *Fishes* (available at http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp) for species names not included in ITIS. Citations must be given of taxonomic references used for the identification of specimens. For example, "Fishes were identified by using Collette and Klein-MacPhee (2002); sponges were identified by using Stone et al. (2011)."

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Word usage and grammar that may be useful are the following:

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Fish and fishes For papers on taxonomy and biodiversity, the plural of *fish* is *fishes,* by convention. In all other instances, the plural is *fish.*

Examples: The fishes of Puget Sound [biodiversity is indicated];
The number of fish caught that season [no emphasis on biodiversity];
The fish were caught in trawl nets [no emphasis on biodiversity].

The same logic applies to the use of the words *crab* and *crabs, squid* and *squids,* etc.

Sex For the meaning of male and female, use the word *sex*, not *gender.*

Participles As adjectives, participles must modify a specific noun or pronoun and make sense with that noun or pronoun.

Incorrect: *Using the recruitment model, estimates of age-1 recruitment were determined.* [Estimates did not use the recruitment model.]

Correct: *Using the recruitment model, we determined age-1 estimates of recruitment.* [The participle now modifies the word *we,* those who were using the model.]

Incorrect: *Based on the collected data, we concluded that the mortality rate for these fish had increased.* [We were not based on the collected data.]

Correct: *We concluded on the basis of the collected data that the mortality rate for these fish had increased.* [Eliminate the participle and replace it with an adverbial phrase.]

Equations and mathematical symbols should be set from a standard mathematical program (MathType) or tool (Equation Editor in MS Word). LaTex is acceptable for more advanced computations. For mathematical symbols in the general text (α, χ^2, π, $\pm$, etc.), use the symbols provided by the MS Word program and italicize all variables. Do not use photo mode when creating these symbols in the general text.

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